Epigenetic effects of maternal prenatal stress on offspring: A systematic Review

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Tiivistelmä – Referat – Abstract

Background and objectives: Epigenetics is the study of changes in gene function without affecting the DNA sequence. Epigenetics studies the effects of the environment and behavior on the genome. Researchers have been able to detect several epigenetic modifications such as – DNA methylation, histone acetylation and microRNA associated gene silencing. Changes in the epigenome are essential for proper cell function and normal development and can also be induced by environmental factors. Stress is defined as a biological response to physiological and psychological demands which can affect cellular homeostasis. Factors such as prenatal life stress can affect gene function without directly altering the DNA nucleotide sequence. Elevated levels of stress can immobilize with the ability to impair cognitive function. There is evidence that suggests the involvement of epigenetic regulation in disorders such as addiction, depression, schizophrenia, and cognitive dysfunction. Therefore, this systematic review discusses recent findings about the role of epigenetics in prenatal exposure to stress. To achieve this, the thesis will cover different subtopics from genetics, neurobiology and diseases, neuroscience, biological psychiatry, life sciences, medicine, behavioral brain research, biochemistry & molecular biology, as well as neuroendocrinology. Research questions are: 1) Is there an association between epigenetics and prenatal stress? 2) What kind of mechanisms have been found? 3) What kind of techniques have been used in identification of potential epigenetic mechanisms? What genes are associated with these epigenetic changes?

Methods: This study followed "The Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) guideline checklist. Eligibility criteria and search terms where be selected and documented to offer the widest range of articles covering the subjects of this study. Literature search was done using PubMed/Medline, google scholar and gray literature. The last sample comprised 59 articles. Data was extracted so that the participants, intervention, comparisons, and outcomes were included.

Results: The literature search conducted in this systematic review identified a few findings. First is that the majority animal and human studies found a significant or moderate association between epigenetics and prenatal stress. Second, DNA methylation is the most studied epigenetic mechanism in maternal exposure to stress Third, genome-wide studies were more common in human studies than in animals and the most widely used method used is Infinium HumanMethylation450 Bead Chip. However, the common methods used in human and animal studies is most likely because of the small sample size and causation cannot be determined. Finally, *NR3C1* and *FKBP5* genes were the most studied in human studies where they showed the strongest association between prenatal stress and epigenetic modifications. While, *i*n animal studies, the most studied genes were *Bdnf* and *Dnmt1* as they showed a significant methylation levels after of maternal prenatal stress exposure.

Conclusions: In conclusion, maternal prenatal stress could trigger epigenetic alterations in neonates in both animals and humans. This holistic review detailed and evaluated locus-specific and of studies exploring current knowledge about associations between maternal prenatal stress and in epigenetic changes.

Avainsanat – Nyckelord – Keywords
Epigenetics, prenatal maternal stress, maternal stress exposure epigenomics, prenatal stress, DNA methylation, histone modifications, non coding RNA

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Table of contents

Abbreviations used in this study in order of appearance:

PS: Prenatal stress
HPA system: hypothalamic-pituitary-adrenal system
11β -HSD2: hydroxysteroid 11-Beta Dehydrogenase 2
GRs: Glucocorticoid receptors
FKBP5: FK506 Binding Protein 5
BDNF: Brain derived neurotrophic factor
GAD1: Glutamate Decarboxylase 1
PN: Paraventricular Nucleus
CRH: corticotropin-releasing hormone
ACTH: adrenocorticotropic hormone
DNMTs: DNA methyltransferase
HDACs: histone deacetylase
HATs: histone acetyltransferase
HMTs: histone methyltransferase
HDMs: histone de-methylases
TFs: transcription factors
ncRNA: non-coding RNA
miRNA: micro RNAs
siRNA: short interfering RNAs
piRNAs: piwi interacting RNAs
5mC: 5 Methyl-cytosine
PCR: polymerase chain reaction
MSP-PCR: methylation-specific PCR
HM450K: HumanMethylation450
NGS: next-generation sequencing
WGBS: whole-genome bisulfite sequencing
MBD: methyl CpG-binding domain
MeDIP: Methylated DNA immunoprecipitation
TET: ten-eleven translocation
H3K4me: tri-methylation at the 4th lysine residue of the histone H3 protein
qRT-PCR: quantitative real-time PCR
lnRNA: long non-coding RNA
HITS-CLIP: High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation
LIGR-seq: ligation of non-coding RNA sequencing
CHART: Capture Hybridization Analysis of RNA targets
CHIRP: Chromatin isolation by RNA purification
RIP: RNA immuno-precipitation
RAP: RNA antisense purification

Introduction

1.1 Prenatal stress

Prenatal stress (PS) studies suggest that long-term exposure to stress throughout prenatal periods can lead to behavioral and developmental changes in offspring (Zhou et al., 2017). Stress makes people more vulnerable to cognitive disorders, but the underlying mechanisms are unknown (Carboni et al., 2010, Tsankova et al., 2006). The role of prenatal stress has been studied intensively in the hypothalamic-pituitary-adrenal (HPA) system development and hippocampus. The psychological facets of stress, such as the random nature of environmental and maternal behavior, have the greatest impact on the progeny's cognitive behavior. Prenatal stress, through altering pruning and development of synapses, can interfere with the normal development of the neural pathways that underpin cognition. Since prenatal stress can be unpredictable and the product of complicated genetic and environmental interactions, determining how certain prenatal life stressors can contribute to the pathology of various psychiatric disorders in human beings has been a significant challenge. Previous reviews have discordant results and that is in part because of the variability of prenatal exposure to stress. Not everyone who experiences stress develops psychological or physiological changes throughout their life, some might develop endurance or resilience (Cheong, Sinnott, Dahly & Kearney, 2017). Understanding the underlying processes may help to identify new therapeutic goals for cognitive disorders. Prenatal stress occurs during pregnancy where the mother is subjected to behavioural or physiological stress because of everyday life activities or environmental challenges. Exposure to prenatal stress can lead to neurodevelopmental conditions in early adolescence (Hosseini Sharif Abad and Sabahi, 2014). Early research studies proposed that prenatal maternal stress can be detrimental to the child's psychological and physiological state in early life and adulthood (Oates, 2002). Additional consequences of maternal stress on offspring involve decreased HPA activity, puberty-onset depression, and child asthma occurrence (Van den Bergh et al., 2005; Cookson et al., 2009). Studies have shown that disruptions in the maternal hormonal stress system are transferred to the fetus via the placenta. As a result, placental abnormalities will impair the growth and development of offspring, ultimately impairing cognitive development and functional stability (Bronson and Bale, 2016). Prenatal stress can trigger preterm labour, which has been associated with cerebral palsy, developmental disorders, and low birth weight (Black et al., 2013, Abedi et al., 2017). According a survey, PS is was estimated to affect 33% to 37% of individuals in the U.K. and about 5% to 7% in Sweden (Woods et al., 2010).

During pregnancy, maternal stress hormones can affect the brain growth of the fetus and the development of the uterus, thus resulting in detrimental effects. The adverse consequences may result from multiple factors such as, the mother's stress level and the pregnancy itself, as well as the fetal environment. Researchers hypothesize how the fetus can have certain, though not all, defense mechanisms such as early life programming because of maternal stress hormone exposure (Welberg LA; Seckl JR, 2012). One example is placental *hydroxysteroid 11-Beta Dehydrogenase 2 (11β -HSD2*) which is hypothesized to have protective effects on the fetus from maternal *glucocorticoid (GR)* levels which has harmful effects on development (Meyer, 1985). When the *11β -HSD2* gene is non-functional because of certain factors, many maternal stress hormones can reach the embryo, thus affecting the development and disrupting the growth of the fetus and tissue production (Maccari, 2010). An animal study in rats has shown that when a disrupted gene function of placental *11β -HSD2* was induced, the rats exhibited a higher incidence of hypertension and hyperglycemia in later life (Govindaraj et al., 2017). Prenatal depression has been shown to be linked with higher risks of developing hypoglycemia, hypertension, hyperlipidemia, and diabetes, as well as other health complications (Lindsay et al., 1996). Other studies also attributed this hypertension and hypoglycemia to low birth weight and prenatal stress (Lima et al., 2018). As a result, the study suggested that prenatal stress could be a link between these disorders and low birth weight (Guzeloglu-Kayisli et al., 2021). Educating people about the dangers of prenatal stress and the physical consequences of stress may help to reduce these risks. The level of stress during a woman's pregnancy can influence brain growth and can predispose the offspring to develop a dysregulated stress response system (Welberg and Seckl, 2014, Coussons-Read, 2013).

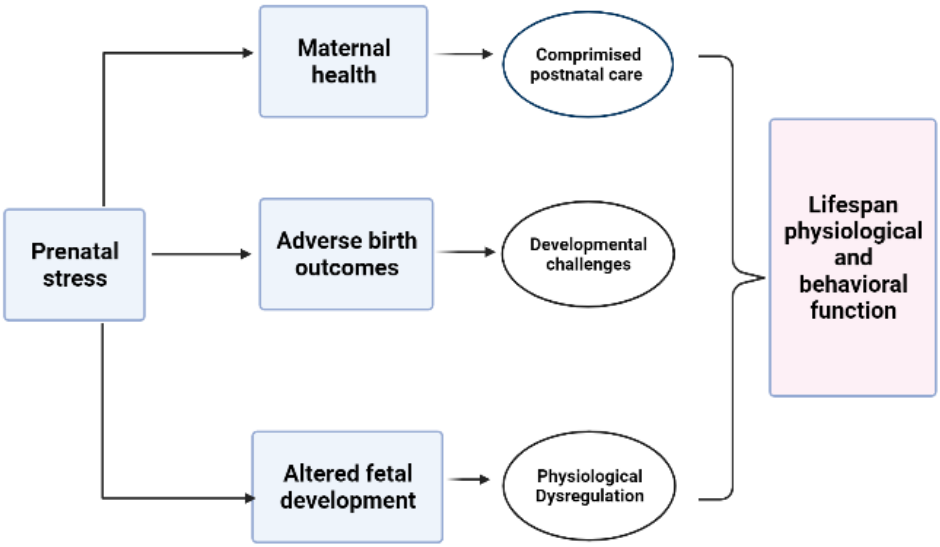

Figure (1) Schematic representation of the pathway of Prenatal stress and its effects on physiology and behaviour (modified from Coussons-Read, 2013).

Prenatal stress has been associated with neurogenesis disturbances throughout adulthood. Rat pups that are subjected to prenatal stress exhibited irreversible behavioral and neurobiological disorders (Aguilera, Fernández, Muñoz, & Fraga, 2010). They also displayed alterations in glutamatergic neurotransmission, glucocorticoid receptors, and adhesive proteins (Maccari 2010). Throughout the end of the breastfeeding period, any momentary stress that is experienced by the mother can have permanent effects on the offspring's neurodevelopment (Vidal et al., 2014). Studies have found that stress exposure during pregnancy can affect neurogenesis in rodents and primates and hinder their ability to grow hippocampal neurons later on (Leuner & Sabihi, 2016). It is unclear which pathways cause long-or maintain slow neurogenesis during postnatal growth, but cortisol is involved (Glasper, Morton, & Gould, 2010). In terms of pathways, likely, elevated corticosterone levels and disturbed maternal behavior in response to prenatal stress correspond to the permanent effects manifested in the fetus through epigenetic changes (Maccari, 2010, Vidal et al., 2014).

1.2 **Epigenetics of Prenatal stress**

Prenatal stress is defined as a pregnant mother's exposure to psychological or physical stress, which can be induced by ordinary life situations or environmental changes (Liu, Erdei, & Mittal, 2021). Because of hormonal changes, genetic and environmental factors, pregnant women experience high sensitivity to emotions, it is estimated that around 10-20% of women experience psychological issues during the late gestation (Brown et al. 2009). Studies have recently revealed that changes in methylation levels on promotors of serotonin transporter genes and glucocorticoid receptors, besides enhancers, have been linked to prenatal maternal susceptibility to depression which is manifested in the DNA of the fetus (Nemoda & Szyf, 2017). However, the underlying molecular mechanisms are still unclear, and more research is needed to further understand the connection between environmental stress, epigenome, and genome. It is hypothesized that epigenetic modifications can alter the offspring's stress system through prenatal exposure to stress through the placenta or other mechanisms (Jensen Peña, Monk, & Champagne, 2012). Prenatal stress has been shown to cause increased DNA methylation in the placenta at various cytosine 5′ to guanine, separated by a phosphodiester bond (CpG) region in the *11β-HSD2* gene promoter (Jensen Pea, Monk, & Champagne, 2012). Because the immune system is so important in the stress response, it is no surprise that some studies have documented multiple immunoregulatory genes that have been methylated under stress, such as *FK506* Binding Protein 5 *(FKBP5),* which is associated with the glucocorticoid pathway and is important in arousal in newborns (Paquette et al. 2014). A study has revealed that during prenatal stress exposure, FKBP5 was highly methylated, which could predict the consequences in newborns (Monk et al., 2016). Another animal study has documented highly methylated genes (i.e., Brain-derived neurotrophic factor (*BDNF*) and glutamate decarboxylase 1 *(GAD1*) that are crucial to the development of the prefrontal cortex, hippocampus, hypothalamus under the influence of prenatal stress (Dwyer & Ross, 2017). Another study proposed that the placenta is important in mediating prenatal stress and its effects on the fetus (O'Donnell et al. 2009; Janssen et al. 2016). Along with this suggestion, animals (Mairesse et al. 2007; Jensen Pena et al. 2012) and human studies (O'Donnell et al. 2012; Blakeley et al. 2013; Reynolds et al. 2015) have shown the association between prenatal stress and disrupted function of the placenta. Since the central organ responsible for behavioral and physiological responses to life stressors is the brain, it also contributes to pathophysiology when it is overburdened and overused. Long-lasting effects on the brain can be caused through epigenetic mechanisms after a stressful event (Ladd et al., 1996; Meany et al., 1989; Plotsky and Meaney, 1993). It is hypothesized that early life stress alters brain structure by affecting synaptic pruning, synaptic overproduction, receptors, and neurogenesis

(Teicher et al. 2006). Epigenetic changes caused by early life stress are often seen on regulatory chromatin sites. Glial cell functioning and synaptic plasticity are affected by epigenetic changes that are caused by early life stress (Hamilton & Rhodes, 2015). It is hypothesized that these changes lead to large disturbances of neuronal networks in neuropsychiatric disorders. The underlying genetic and epigenetic mechanisms are still unknown, which makes diagnosis exceedingly difficult (Pechtel and Pizzagalli, 2010). Research in prenatal stress exposure has shown a strong connection to several psychiatric diseases, including severe depression, post-traumatic stress disorder, and major bipolar disorder (Babicola et al., 2021). Stress is a complicated process that manifests in a broad range of physiological effects, ranging from epigenetic changes to inflammatory responses to a dysregulated hypothalamic-pituitary system, therefore complicating the role of discovering biomarkers could possibly be correlated with psychiatric disorders (Syed & Nemeroff, 2017). Although the mechanisms underlying stress and its long-lasting effects on cognitive function are still not clear, evidence has shown a potential role for epigenetic modifications (Weaver et al., 2005). Epigenetic regulation prepares genes for future environmental responses which allow adaptation to variations in environmental conditions (Eckhardt et al, 2006). In cases of differences between early and later life environments, this adaptive epigenetic response can increase pathological risks. Several human and rodent studies have shown that phases of the early environment can cause intense physical and mental developmental changes which can lead to altered cognition, behavior, and mood (Song et al., 2012). Epigenetics is the study of gene expression changes that occur throughout development and are influenced by the environment (Berger et al., 2009). The most studied epigenetic modifications that are involved in regulating dynamic gene expression throughout stress are DNA methylation and histone modifications which help pack the DNA into chromatin (Tsankova et al., 2007). DNA Accessibility is controlled by epigenetic modifications which regulate gene expression. Genes that are accessible get transcribed but in inaccessible genes are silenced (Szyf et al., 2005). This process enables the combination of the environmental entrance to the genome, which facilitates adaptation (McGowan et al., 2009). The chromatin state is highly influenced by letters such as maternal care and stress. Gene activation and inactivation alter protein production, which can affect behavioral and physiological states; over time, these changes accumulate within the organism, causing cellular changes that can be passed down from parent to offspring (Roth et al., 2009) and therefore, contribute to changes in arginine vasopressin and corticotropin releasing hormone expression levels due to stress. Epigenetic modifications mediate fundamental stress response elements (Stankiewicz, Swiergiel, & Lisowski, 2013). Meaney's groundbreaking work (Meaney, 2001) was the first to show that maternal care affects the methylation level of offspring.

The impact of early life and environmental stressors on methylation levels of glucocorticoid genes was investigated by (Weaver et al., 2004) where it was shown that *GR* receptors are feedback modulators of the HPA system as they are essential to maintaining a homeostatic response to stress (Tsigos and Chrousos, 2002). One study (Francis and Meaney, 1999) showed that adult rats who were deprived of maternal care (i.e. grooming and nursing) as pups had low hippocampus concentrations of *GR* and high stress levels. Another behavioral study using maternal separation in rodents (Kember et al., 2012) reported male pups that were subjected to maternal deprivation showed cognitive impairment when exploring a maze which was induced by high methylation levels of the *CRH* (corticotropin-releasing hormone) gene that handles stress response regulation. According to Own et al. (Own et al., 2013), maternal separation in mice pups is also associated with enhanced *SLC6A4* (serotonin transporter) gene methylation levels, *SLC6A4* gene is a modulator of the serotoninergic system in the stress response (Lesch 2011). Young adults who have been subjected to prenatal and postnatal stress had similar epigenetic modifications (Provenzi et al., 2016). The two main pathways in humans that are highly susceptible to epigenetic changes were found to be the serotoninergic pathway and the HPA system (Vreeburg et al., 2009). The effects of childhood trauma on brain health are intensively studied and there are various mechanisms that link trauma-related inflammation to the development of brain health (Dranovsky et al., 2011,Howren et al., 2009).

1.2.1 The HPA system

The HPA axis is triggered by stress throughout development which can generate long-lasting molecular and cellular hippocampal changes, consequently resulting in neurobehavioral changes later in life (Cuadra et al., 1999, Dunn and Swiergiel, 2008, Gardner et al., 2009). When an individual encounters a stressful event, the autonomic nervous system triggers a "fight-or-or-flight" reflex". Therefore, the HPA axis must be tightly controlled to maintain stress mechanisms under balance. Areas in the brain such as the paraventricular nucleus (PN) react to a stressor through the release of neuropeptides, arginine vasopressin (AVP), and corticotropin-releasing hormone (CRH). The anterior pituitary gland regulates growth, lactation,

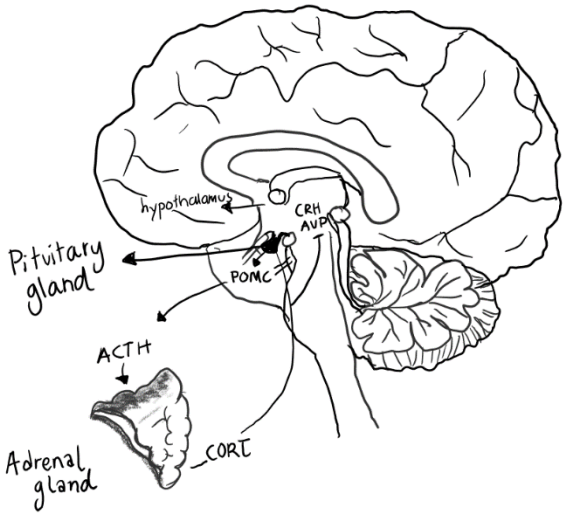

Figure (2) The HPA stress system. . In the parvocellular neurons of the paraventricular nucleus, the neuropeptides corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) are produced. The simultaneous release of CRH and AVP into blood vessels stimulates anterior pituitary adrenocorticotropic hormone (ACTH) secretion and adrenal corticosteroids. The glucocorticoid receptor inhibitory effects oppose the HPA axis impact on the anterior pituitary gland, hippocampus, and hypothalamus. Image modified from https://www.healthrising.org/blog/2020/06/18/cortisol-fibromyalgia-chronic-fatigue-syndrome-neuroinflammation/

and reproduction. It releases adrenocorticotropic hormone (ACTH) to produce cortisol in humans and animals. AVP is a growth factor, antidiuretic, and produces peripheral vascular vasoconstriction. It also involves cognition, tolerance, adaptability, and complicated sexual and maternal behaviour, and regulation of cardiovascular and water excretion (Salata, Jarrett, Verbalis, & Robinson, 1988).

When the stress mechanism is no longer activated, a negative feedback loop through *GR* guarantees a homeostatic balance (Fig. 2). Negative feedback systems may become downregulated with acute stress and abnormal HPA activation is one of the most often noted neuroendocrine signs of severe depression. Holsboer, 2000). Prolonged or chronic stress in early childhood leads to increased glucocorticosteroid production by the HPA axis (Gonzalez, 2013). Rodent studies show stressors can have medium-to-long-term effects on HPA activity and behavior by modifying neuropeptide expression (Kall, Just & Aschner, 2016). Herman et al. (2003) argues that areas in the limbic system cause a predictive stress response which is critical for regulating the HPA axis.

1.2.2 Epigenetic mechanisms

1.2.3 DNA methylation

The addition of a methyl group (CH3) on the DNA at a CpG site is referred to as DNA methylation and it plays an important role in gene silencing and gene expression regulation (Irizarry et al, 2009). Therefore, enhancers and promoters that are active are normally not methylated but become inactive once they are methylated (Moore et al., 2012). DNA structure and function are changed once a methyl group is inserted (McCoy et al., 2016). Two outcomes are likely to occur when DNA and a methyl group are attached: 1) DNA binding of transcription factors are directly blocked or 2) interference with transcription factor accessibility is induced by factors that are attached to methylated or unmethylated DNA (Shen et al, 2007). Most CpGs (70-80%) are methylated, and about 85% are found in repetitive sequences known as transposons, which account for roughly half of the human genome (Meissner et al, 2008). And the other 15% are found within Guanine cytosine-rich regions known as CpG islands comprising over 500 base pairs. In transcriptionally active genes, there seems to be a higher methylation level on CpG islands compared to DNA methylated silencing in enhancers and promoters (Sharifi-Zarchi et al., 2017). It could also imply that DNA methylations are involved in regulating transcription. There are three types of DNA methyltransferases DNMTs where each one has a specific function. DNMT1 is a maintenance enzyme that detects DNA that is semi-methylated, and it starts methylating replicated

daughter strands on cytosine sites. De novo methylases are DNMT3a and DNMT3b which target unmethylated DNA (Pradhan et al, 1999).

1.2.4 Histone modifications

The DNA is wrapped around histone proteins to form nucleosomes which fold into compact chromatin (Martin et al., 2004). There are two major ways through which histone modifications occur. The first one includes direct chromatin structure modifications. The second one involves the regulation of effector binding molecules (Bannister & Kouzarides, 2011). The key indicator for an active chromatin form is histone acetylation, while histone methylation can either activate or silence genes. Chromatin remodelling would not be deemed possible without specific regulating enzymes involved. These marks are deposited on the histone tails by specific enzymes that modify histone such as histone deacetylases (HDACs), histone acetyltransferases (HATs), histone methyltransferases (HMTs), and histone demethylases (HDMs) (Unnikrishnan et al., 2010). HATs change the N terminal of histone proteins by adding an acetyl group and HDACs remove an acetyl group. HMTs methylate histone tails while HDMs remove methyl groups from histone tails (Dion et al., 2005). These modifications are essential for chromatin remodeling. When chromatin is in an accessible open state, it is referred to as euchromatin, while the closed densely packed form is known as heterochromatin (Jenuwein and Allis, 2001). Recruitment of histone enzymes is accomplished by transcription factors (TF) interactions which detect and bind to specific gene sequences (Alyamani & Murgatroyd, 2018).

1.2.5 Noncoding RNA

Studies have shown that about 2% of the human genome is translated into proteins while the rest is only transcribed. Some of these non-translated parts of the genome are known as non-coding RNA which function in gene expression regulation such as posttranslational and transcriptional modifications. Noncoding RNAs are separated into two further groups, which are long ncRNAs that are over 200 nucleotides and short ncRNAs which are less than 30 nucleotides long. Short ncRNAs have 3 dominant classes, which are micro RNAs (miRNAs), short interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs). Both major groups have been shown to regulate gene silencing, histone modifications, formation of heterochromatin, and DNA methylation (Denis, Ndlovu & Fuks, 2011) (Yuan et al., 2011).

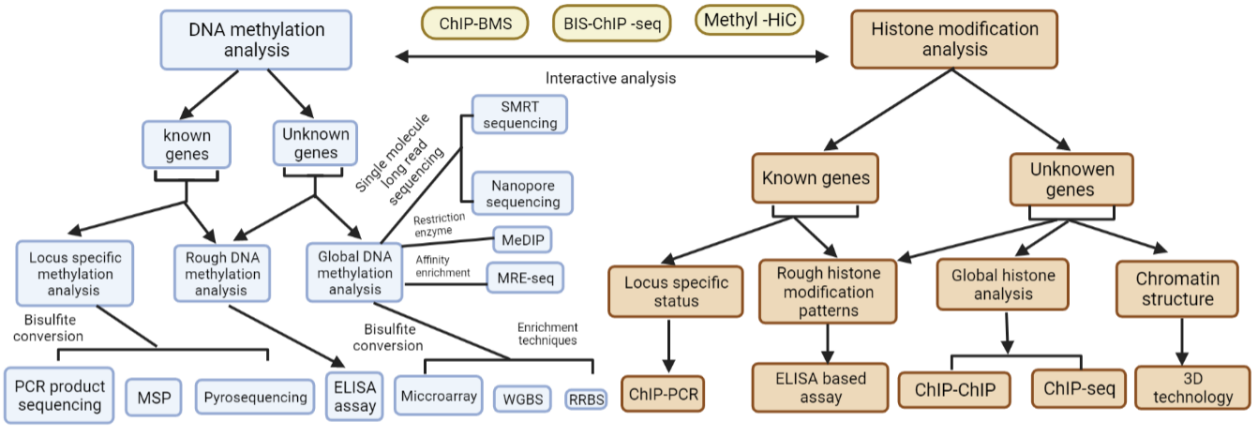

Figure (3) An algorithm-generated illustration for selecting suitable methods of various epigenetic codes. The methods used to analyse DNA methylation 5mC (blue), histone modifications (orange) varied according to the design and purpose of the study.

Image modified from (Li, 2021) using biorender.

1.3.1 DNA methylation methods

A) Locus specific DNA methylation methods

As technological advances have boomed over the past 20 years, methods of DNA methylation detection at different levels and dimensions have been well established. Therefore, it is vital to choose the most suitable method to use when trying to answer a specific research question. The DNA bisulfite procedure, invented in the early 1900s, is widely regarded as an indispensable method for determining the methylation state of the DNA (Tang, 2019). Bisulfite Conversion is a procedure in which genomic DNA is denatured and treated with sodium bisulfite. This method allows the removal of cytosine residues and leaves 5-methylcytosine residues intact which allows 5mCs to be separated from non-methylated cytosines (Leontiou et al., 2015). Bisulfite treatment-based DNA methylation analysis enables the identification of 5mC at single specific regions on the DNA. Several methods have been suggested for detecting DNA methylation levels that are unique to a particular locus, such as subcloning sequencing, direct bisulfite polymerase chain reaction (PCR) sequencing, pyrosequencing analysis, and methylation-specific PCR (MSP-PCR). The MSP method uses DNA that is already bisulfite converted into a probe for consequent PCR procedures for the determination of locus-specific methylation status by using forward and reverse primers to distinguish between methylated and

unmethylated DNA. Pyrosequencing-based DNA methylation analysis can provide reproducible and precise quantification of the methylation status of a gene of interest with high quantitative resolution following amplification using PCR. (Li, 2021). Pyrosequencing is considered an effective, rapid, and accurate method that can process about 96 bisulfite-converted DNA samples in approximately 4 hours every run. Pyrophosphate is released upon every incorporated and added nucleotide, then is converted into a light signal through luciferase. (Zhao & Bapat, 2016). Pyrosequencing allows measuring the methylation status of CpG regions of a PCR resulting product which is generated after bisulfite treatment through primers that bind to either methylated or unmethylated regions. It is based on the primer extension and the release of pyrophosphate which undergoes bioluminometrical quantification through a Pyrosequencer such as (QIAGEN) (Hattori & Ushijima, 2011).

B. Genome-wide profiling of DNA methylation

DNA methylation can lead to dynamic, sequence-dependent, and trans-generationally heritable changes in the DNA methylation patterns of tissues (Yong et al., 2016). The most common experimental techniques used in genome wide profiling constitute bisulfite conversion, affinity enrtichment and enzyme digestion figure (3) and figure (4) (Laird, 2010). Therefore, the distribution of methylated genes in the DNA can help enhance our understanding of important complex biological processes. Genome-wide detection of DNA methylated genes is made possible through first, reduced representation bisulfite sequencing, hybridization-based microarray (Illumina HumanMethylation450-HM450K) theHM450K device can accommodate over 450,000 CpG methylated regions, EPIC Illumina microarray with approx. 900 000 is the latest , or high throughput next-generation sequencing analysis (NGS) as whole-genome bisulfite sequencing (WGBS) (Peters et al., 2015).

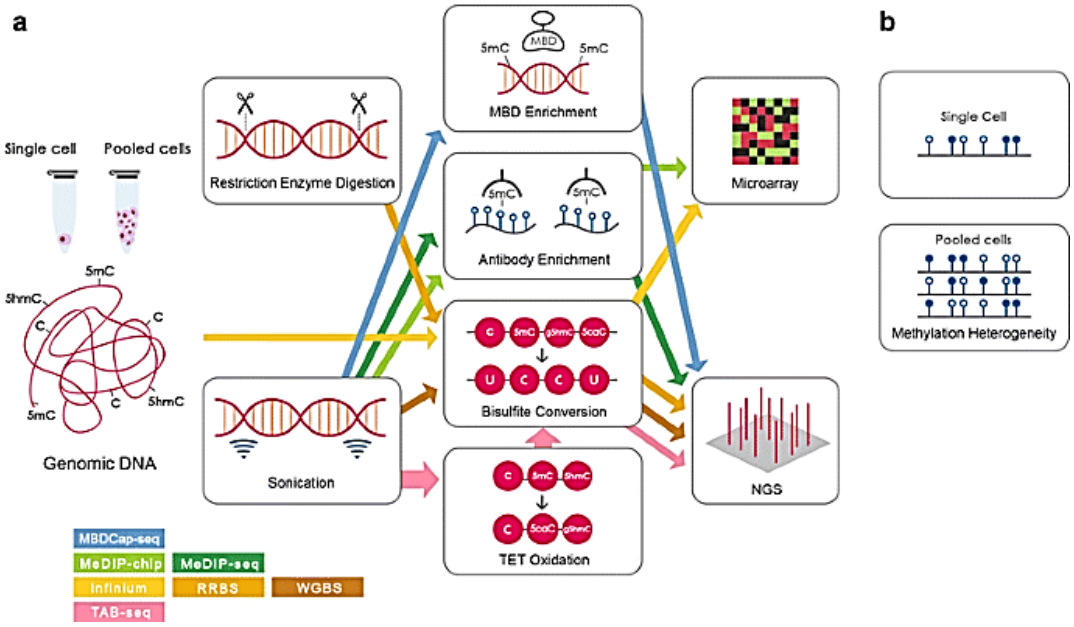

Figure (4) The most frequently used techniques for analysing DNA methylation at the genome-wide level.

A. The procedures can require fragmenting genomic DNA using restriction enzymes or ultra-sonication. Before testing the genomic DNA using a microarray or next-generation sequencing tool, the DNA may be exposed to bisulfite conversion, antibody enrichment, methyl CpG-binding domain proteins (MBD) enrichment, or ten-eleven translocation family of proteins (TET) oxidation.

B. Single-cell DNA methylation study, which requires the isolation of individual cells, enables the estimation of heterogenous methylated cell populations, while other genome-wide methylation methods which use pooled cell heterogeneous populations can not distinguish diverse methylated regions. 5mC is represented by blue concrete circles, while Cytosine is represented by hollowed concrete dots. The image got from (Yong, Hsu, & Chen. 2016) (Yong, Hsu, & Chen, 2016).

1.3.2 Epigenetic Methods used for histone modifications.

To determine histone modifications on the DNA, it's crucial to look at the number of modifications present, and regulatory proteins such as transcription factors. To identify histone modifications, chromatin is often cross-linked to specific antibodies for the targeted cation such as tri-methylation at the 4th lysine residue of the histone H3 protein (H3K4me) and then the changed fragments immune-precipitate which are eventually collected for preparation of a sequencing library. Later, chromatin immunoprecipitation next-generation sequencing (ChIP-

seq) uses DNA molecules to detect the genomic region of a protein. This same principle can apply to localizing variants of histone modifications (Zhang et al., 2005; Barski et al., 2007). ChIP is the most conventionally applied method for the quantification of chromatin interaction patterns and modifications. Figure (5, B). The ChIP assay uses antibodies that detect certain histone alteration markers of epigenetic modulators in combination with specific DNA fragments, allowing locus-specific roles of post-translational modifications or transcriptional factor complexes to be assigned. ChIP accompanied by traditional PCR or quantitative real-time PCR (qRT-PCR) will show enrichments of specific histone modifications or the binding capacity of a remodeling complex to a specific DNA regulatory region Figure (5, B). when the modifications aren't specified, sequencing-based ChIP approaches like ChIP-chip or ChIP-seq may analyze any events with protein to DNA binding and histone modifications that take place at a wide range of loci at the same time (Deangelis, Farrington, & Tollefsbol, 2008).

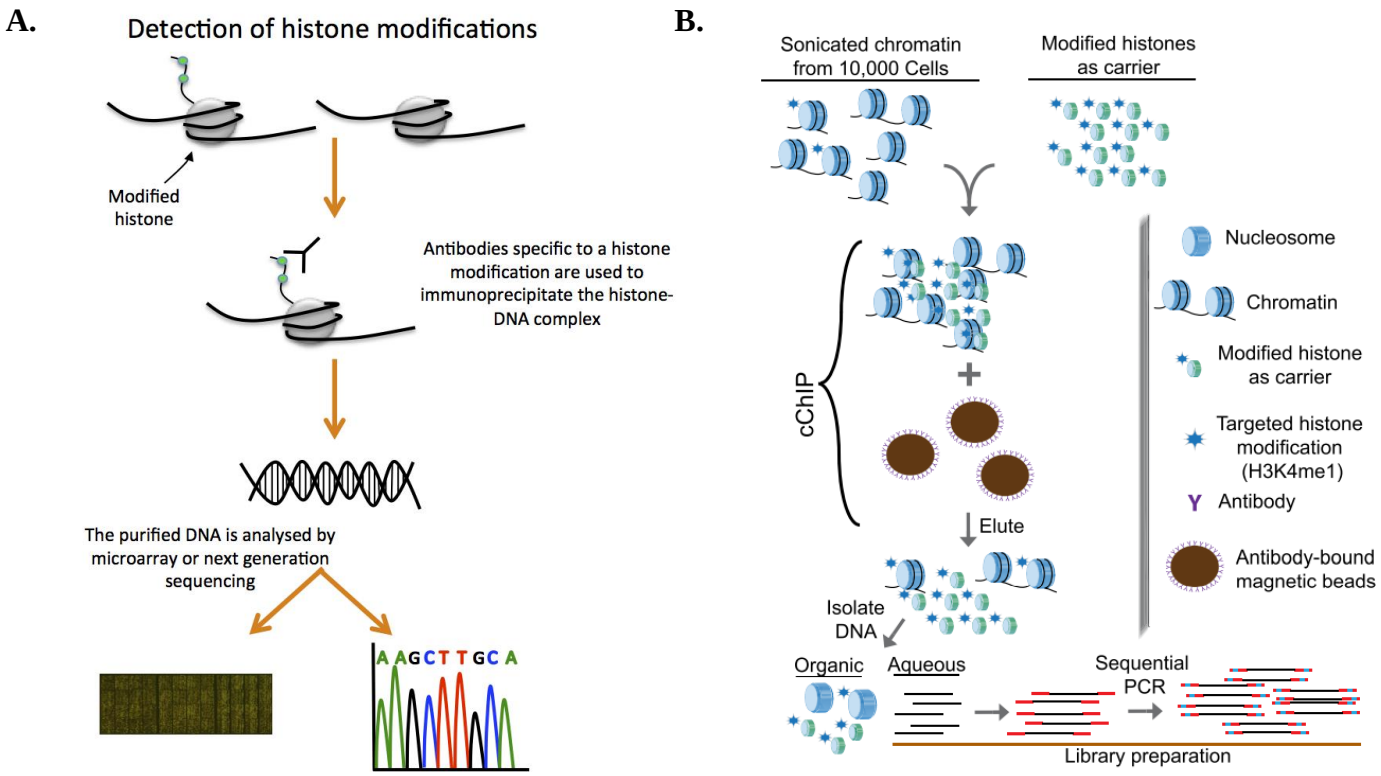

Figure (5) Profiling of Histone modifications

A. Techniques used to detect histone modifications include Immunoprecipitation methods which are accompanied either with NGS or Microarrays (Kimura, 2013)

B. B. ChIP-sequencing technique illustration. (Valensisi, Liao, Andrus, Battle, & Hawkins, 2015)

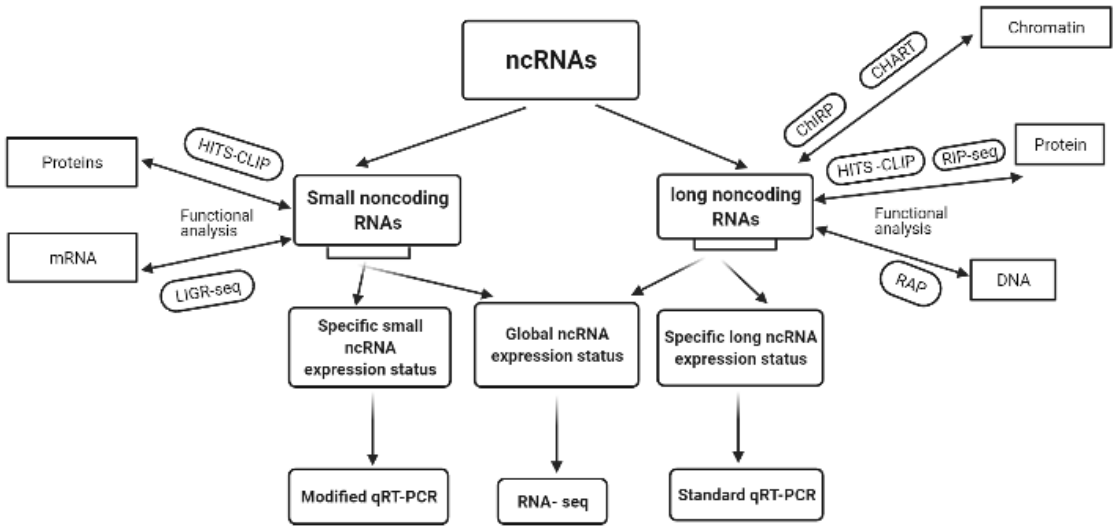

Figure (6) Noncoding RNA epigenetic profiling

RAP: RNA antisense purification, ChiRP: Chromatin Isolation through RNA Purification, qRT-PCR: quantitative real-time polymerase chain reaction, CHART: Capture hybridization analysis of RNA targets, RIP-seq: RNA immunoprecipitation sequencing, HITS-CLIP: High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation, and LIGR-seq: ligation of noncoding RNAs sequencing. Image modified from (Li, 2021) using biorender.

1.3.3 Epigenetic methods used for ncRNAs

Next-generation approaches that perform whole transcript amplification have increased the specificity and sensitivity of ncRNA identification at single-nucleotide resolutions because of their lack of need for target probe hybridization which is often used in microarray (Jung et al., 2010) (Zhou et al., 2011). Most methods that isolate RNA often use long noncoding RNA (lncRNAs) in transcription experiments, while for short ncRNAs, amplification and extraction are usually necessary.

A. Techniques used for small ncRNAs

Noncoding RNA quantifying methods are now well developed which include analysis using (qRT-PCR), hybridization microarray, and high throughput next-generation RNA sequencing. Real-time PCR uses commercial kits that have transcription primers that are stem looped for 3 'templates which provide accurate, simple, and sensitive ncRNA quantification. The ability of noncoding RNAs to function with various proteins, transcription factors, DNA, and RNA provides a major advantage for high throughput sequencing. A method that has been used to identify functional protein-RNA interactions is high-throughput sequencing of RNA isolates through crosslinking immunoprecipitation (HITS-CLIP).While interactions between microRNA and mRNAs are profiled using ligation of non-coding RNAs (LIGR-seq) can be used as potential targets and biomarkers for various pathologies.

B. Techniques for long ncRNAs

Long noncoding RNAs are usually over 200 bp. Poly (A) tail presence plays a crucial role in lncRNA isolation (Bertrand-Lehouillier, Legault, & McGraw, 2019). LncRNAs can be identified using standardized quantitative RT-PCR only if they have a poly (A) tail. Other high throughput methods aim to globally profile long ncRNAs. For example, Capture Hybridisation Analysis of RNA targets (CHART) and Chromatin isolation by RNA purification (ChIRP) are techniques that can be used to identify long non-gene bound regions in DNA.; the RNA immunoprecipitation (RIP) method, RIP-seq, and RIP-chip are used to detect protein interactions with RNA; and RNA antisense purification (RAP) is used to map the location of RNAs, and this technique is often coupled by bioinformatics methods and datasets so that researchers can easily be able to characterize and define specific functions for long ncRNAs. Long ncRNA research methods and applications in biomedicine and clinical settings have the potential to contribute to a better understanding of the potential functions of these molecules in different biological processes. (Deangelis, Farrington, & Tollefsbol, 2008).

1.4 Study questions and hypothesis

Previous reviews have discordant results, and that is in part because of the variability of stress. Not everyone who experiences stress develops psychological or physiological changes throughout their life, some might develop endurance or resilience (Cheong, Sinnott, Dahly & Kearney, 2017). When researchers understand the connection between epigenetics and prenatal stress, they may explain the pathology of many diseases. Understanding the underlying processes may help to identify new therapeutic goals for cognitive disorders. This review is an addition to the body of literature available. By identifying and summarizing the current findings relating prenatal stress exposure to DNA methylation, histone modifications, and non-coding RNA modifications, it is expected to find that there is a strong association between epigenetic modifications and in utero exposure to psychological (Murgatroyd & Spengler, 2011).

The research questions are:

Is there an association between epigenetics and prenatal stress? t has been hypothesized that the development of epigenetic mechanisms that regulate the expression of genes during early life stages can also contribute to the phenotypic consequences of stress. (Szyf, 2019).

What kind of epigenetic mechanisms (i.e., DNA methylation, histone modifications, and noncoding RNA) have been studied and known to be involved in prenatal stress exposure, and how much is currently known about the mechanisms? It has been proposed that prenatal and postnatal environmental conditions can trigger DNA methylation changes which can facilitate epigenetic programming of crucial stress regulating genes which could manifest with neuroendocrine and behavioral symptoms throughout adulthood (Murgatroyd & Spengler, 2011).

What methods and studies are currently being used in epigenetics (i.e., locus and genome-wide association studies), and what are the genes involved that are being studied that relate to prenatal stress and epigenetics?

2. Methods

2.1. Protocol

The protocol of this systematic review followed "The Preferred Reporting Items for Systematic reviews and Meta-Analysis" (PRISMA) when applicable (Liberati et al., 2009). Eligible criteria, search strategy, study selection, data collection, and evaluation of limitations and biases were defined and performed according to PRISMA under the PICo protocol for qualitative reviews. Prenatal stress was defined to be inclusive of only psychological stressors.

2.2. Eligibility criteria

Studies were included from (1974-2021) with the following inclusion requirements: a) papers presented in peer-reviewed journals and written in English c) nonduplicate studies d) animal and human studies e) the study included a valid measure of epigenetic changes that were associated with environmental stressors in prenatal periods f) study evaluated if epigenetic changes were correlated with prenatal stress exposure g) Clinical studies relevant to the research questions were included h) Candidate gene studies, locus and genome/epigenome-wide association studies were included i) Articles that showed results from one of the following topics of Epigenetics & prenatal stress: neurobiology and diseases, neuroscience, biological psychiatry, genetics, life sciences, medicine, behavioral brain research, biochemistry & molecular biology, and psycho-neuroendocrinology. This study Followed the PICo protocol: a) Population: Human and Animal Studies that are exposed to prenatal stress, b) Intervention: Analyze the methods used and epigenetic gene changes that are associated with prenatal maternal stress, c) Context: Document different results between human and animal studies and compare the methods used in each study, and d) Outcome. · Articles that were excluded were: a) Systematic Reviews b) Reviews c) Meta-analysis d) unpublished manuscripts and conference abstracts e) Books and documents f) Duplicates g) studies that were not in English h) Studies that did not match the population and epigenetic methods in prenatal stress i) Dissertations were not included.

2.3. Search procedure

This systematic review was based on a literature search which was done on June 4th,2021 using PubMed, Medline, and google scholar to identify the animal and human studies with no period restriction that focused on epigenetics and prenatal stress. The search term was: "Epigenetics and prenatal stress NOT review" with no additional filters. Grey literature was scanned for related articles. To ensure that all-important articles were found, the articles found during the initial search were scanned for valid references, and the gray literature using google scholar yielded 20 extra articles.

2.4. Study selection

The literature search resulted in 394 articles in PubMed/MEDLINE, and additional 20 articles through gray literature by scanning references of related articles. After the removal of duplicates and reviews, altogether 312 records were screened by abstract and title. After this first screening, 206 records were excluded. The 106 remaining records were screened again based on full text, where 47 articles were excluded. Therefore, the last stage included 59 articles in total. A PRISMA (2020) based flowchart for the study selection can be seen in Figure (7) below.

2.5. Data extraction

Fifty-nine articles were included at the end were screened for extraction through the Rayyan website for analyzing systematic reviews and divided into categories animal studies, Human studies, and subcategories based on the methods used: GWAS study or locus-specific study.

2.6. Data analysis

Study selection was limited to human and animal studies. Lastly, a meta-analysis of the relevant literature was not completed for two primary reasons: 1) Methylation levels of DNA were quantified differently across studies (e.g., percent methylation vs. full/no methylation), and 2) the measurement of CpG sites within genes examined in each study was not consistent, making comparisons across studies difficult. Taking these factors into account, a qualitative review provides an efficient review of the literature, and effect sizes are reported when available.

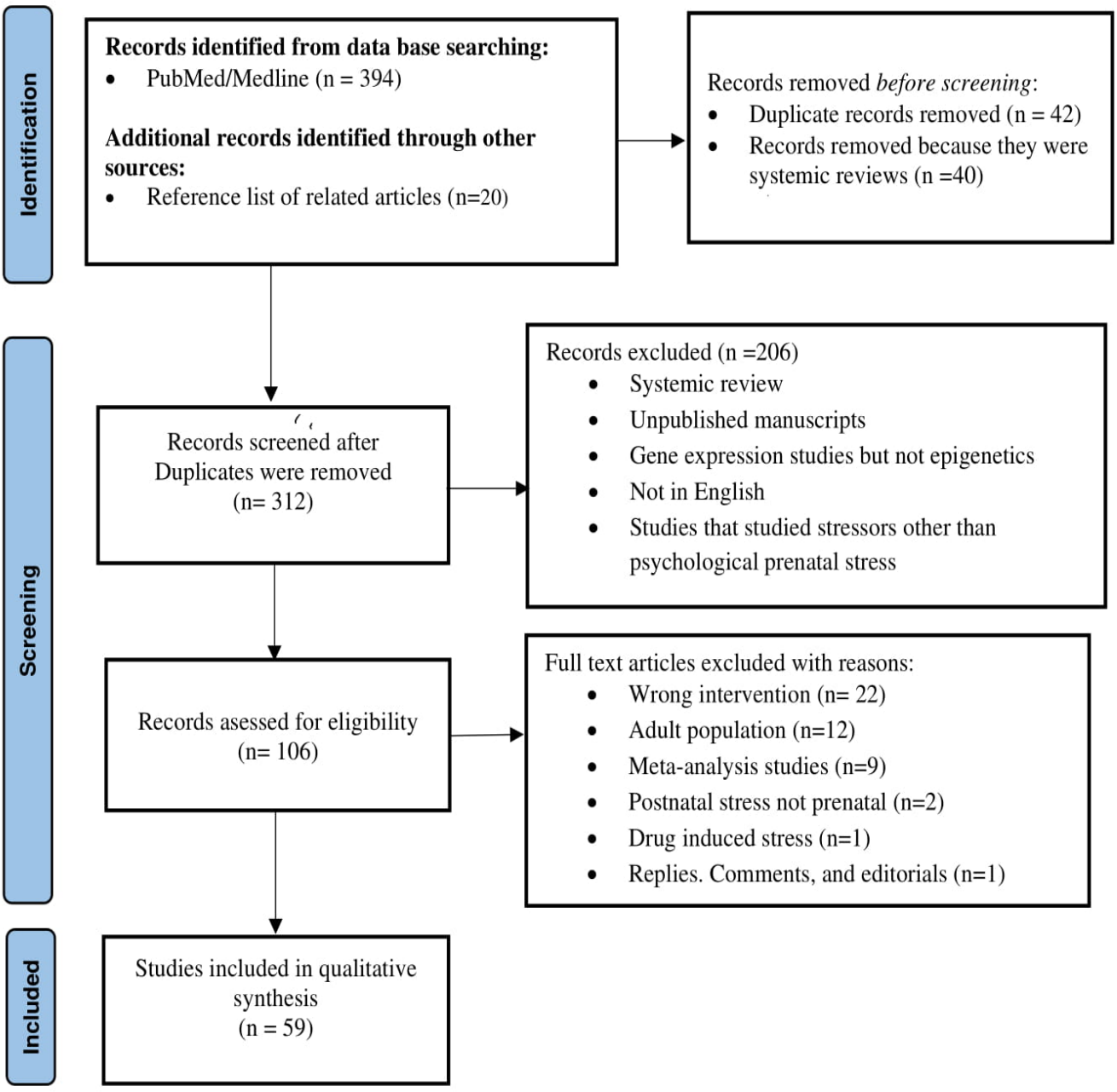

Figure (7) Flow Chart for study selection based on PRISMA guidelines 2020.

3. Results

Table 1 and Table 2 present the 59 full-text articles with study information for each one besides supplementary tables 1 and 2. All the studies focused mainly on psychological stress. Thirty studies were human studies and 29 were animal studies including rat, mouse, and bovine studies**.**

Table 1 Summary of Human studies

Category	Subcategory	Articles	Methods	Candidate genes	Common Associated phenotype
	N=59				
HUMAN	***DNA methylatic***	Total n= 30			
	Locus specific	n= 18	bisulfite pyrosequencing, Sequenom EpiTYPER, MassARRAY and qPCR	*NR3C1 exon 1F , BDNF IV , IGF2, NR3C2, AVP , FKBP5 rs1360780 and intron 7 , HSD11B2, SLC6A4, DRD4 CpG7, OXTR rs53576*	Increased salivary cortisol stress , poor sleeping, prenatal trauma- related symptoms were evident, maternal depression .
	Genome-wide	n= 8	Infinium Human Methylation 450 BeadChip Array, Whole genome bisulfite sequencing (WGBS),	*LTA, NFKBIA, PIK3CD, SCG5 , PC2 , NFKB2 , SLC6A4, CCDC114 , KLF6 , TBR1,IGF2 , igf1 , NMUR1/2 , GNA11, CACNB4 ,PPP3R1 , NFATC3,CRBN , MDFIC and* Methylation at pathways involved zinc finger proteins.	Deregulated neuroendocrine and neurotransmitter receptor interactions were observed in stressed mothers and their children.
	both	n= 1	450K Beadchips and bisulfite sequencing;	DMR in the GABA-B receptor subunit 1 gene *(GABBR1)*	Cord blood GABBR1 methylation was associated with infant cortisol levels in response to a routine vaccination at 4 months old.
	histone modification				
	locus specific	N/A	N/A	N/A	N/A
	genome-wide	N/A	N/A	N/A	N/A
	miRNAs				
	locus specific	N/A	N/A	N/A	N/A
	genome-wide	n=1	Methylated DNA immunoprecipitation (MeDIP) analysis and Gene and miRNA expression analyses by Real Time PCR	miR-30a	miR-30a-5p levels were significantly elevated in the blood of depressed patients who experienced traumatic events early in life, as compared to controls

Abbreviations in order:*NR3C1 (Nuclear Receptor Subfamily 3 Group C Member 1), BDNF (Brain-Derived Neurotrophic Factor), IGF2 (Insulin-Like Growth Factor 2), NR3C2 (Nuclear Receptor Subfamily 3, Group C, Member 2), AVP (Arginine Vasopressin), FKBP5 (FKBP Prolyl Isomerase 5), HSD11B2 (Hydroxysteroid 11-Beta Dehydrogenase 2), SLC6A4 (Solute Carrier Family 6 Member 4), DRD4 (Dopamine Receptor D4), OXTR (Oxytocin Receptor), LTA (Lymphotoxin Alpha), NFKBIA (NFKB Inhibitor Alpha), PIK3CD (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit), SCG5 (Secretogranin V), PC2 (Proprotein convertase 2), NFKB2 (Nuclear Factor Kappa B Subunit 2), CCDC114 (Coiled-Coil Domain Containing 114), KLF6 (Kruppel Like Factor 6), TBR1 (T-Box Brain Transcription Factor 1), IGF1 (Insulin Like Growth Factor 1), NMUR1/2 (Neuromedin U Receptor 1/2), GNA11 (G Protein Subunit Alpha 11), CACNB4 (Calcium Voltage-Gated Channel Auxiliary Subunit Beta 4), PPP3R1 (Protein Phosphatase 3 Regulatory Subunit B, Alpha), NFATC3 (Nuclear Factor Of Activated T Cells 3), CRBN (Cereblon), MDFIC (MyoD Family Inhibitor Domain Containing)*

Table 2 Summary of Animal studies

Category	Subcategory	Articles	Methods	Candidate genes	Common Associated phenotype
ANIMAL	***DNA methylation***	***n=18***			
	locus specific	n=12	Bisulfite pyrosequencing, methylated DNA immunoprecipitation and qPCR, methylated-DNA immunoprecipitation (MeDIP)	*BDNF, DNMT1a, DNMT3a , myelin basic protein (Mbp), BDNF IV , PDLIM5 promoter, TrkB ,CRH*	increased depression-like behavior in the forced-swim test, . PS offspring rats showed a significant decrease in sucrose preference and a prolonged immobility time. PS increased anxiety-like behavior in offspring, especially in females.
	genome-wide	n=6	bisulfite sequencing methods, Methyl-MiniSeq , Methylated DNA immunoprecipitation using MeDIP/seq , ChIP assays, Pyrosequencing , qRT-PCR	*OPRK1, OPRM1, PENK, POMC, NR3C2, TH, DRD1, DRD5, COMT, HTR6, HTR5A, GABRA4, GABRQ, and GAD2, (SLC5A2), (JPH2), (SIGLEC15), (GSC), (IGFBP1), (LRATD1), CXADR (CLMP), Gad1 , Reln (Reelin) and Bdnf, 1675 CGs of 957 immune system genes, CRF and GR*	Elevations in temperament score and serum cortisol through weaning , . In a comparison of the PTSD group versus the control group, 4,160 significantly differentially methylated loci containing 30,657 CpGs were identified, Also found that male offspring exposed to stress early in gestation displayed maladaptive behavioral stress responsivity,
	histone modification	***n=4***			
	locus specific	n=2	EpiQuiK Global Histone H3 Acetylation Assay Kit and ChIP analysis	histone H3 acetylation decrease (p = .06), measured histone H3 trimethyl Lys4 (*H3K4me3*) of *OGT* mRNA, protein, and *O-GlcNAc*	Prenatal stress decreases PFC histone H3 acetylation in males.(O-GlcNAc) transferase (OGT) O-GlcNAcylation, were significantly lower in males and further reduced by prenatal stress.
	genome-wide	n=2	ChIP	Increased accumulations of active histone marks H3 lysine (K) 4me3, H3K14ac, and -H3K36me3	Decreased hippocampal neurogenesis
	miRNAs	***n=7***			
	locus specific	n=2	RT-PCR, pyrosequencing, retrotranscription and qPCR	miR-10b-5p, microRNA-133b	Decreased hippocampal neurogenesis by low numbers of proliferation markers, neuronal differentiation markers and decreased hippocampal BDNF levels. Prenatal stress induced changes in gpm6a levels in both tissues and at both ages analyzed, indicating a persistent effect. microRNA-133b was the most significantly altered.
	genome-wide	n=5	Custom Taqman qRT-PCR Array, Microarray analysis, NGS	miR-322, miR-574-3p,and miR-873, upregulated miR-103 . PNS upregulated miR-145, miR-323 ,miR-98, miR-219, which targets the gene Dazap1, differential expression of 157 miRNAs and 1009 genes, 250 miRNAs, miR-30a and *Neurod1*	Observed a broad shift inexpression from a male-typical to a more female-typical patternin the F2 male offspring of prenatally stressed sires. Offspring transcriptomic changes included genes related to development, axonal guidance and neuropathology.

Abbreviations in order: *DNMT1a (DNA Methyltransferase 1), DNMT3a (DNA Methyltransferase 3 Alpha), PDLIM5 (PDZ And LIM Domain 5), TrkB (Tropomyosin receptor kinase B),CRH (Corticotropin Releasing Hormone), OPRK1 (Opioid Receptor Kappa 1), OPRM1 (Opioid Receptor Mu 1), PENK (Proenkephalin), POMC (Proopiomelanocortin), TH (Tyrosine Hydroxylase), DRD1 (Dopamine Receptor D1), DRD5 (Dopamine Receptor D5), COMT (Catechol-O-Methyltransferase), HTR6 (5-Hydroxytryptamine Receptor 6), HTR5A (5-Hydroxytryptamine Receptor 5A), GABRA4 (Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha4), GABRQ (Gamma-Aminobutyric Acid Type A Receptor Subunit Theta), GAD2 (Glutamate Decarboxylase 2), SLC5A2 (Solute Carrier Family 5 Member 2), JPH2 (Junctophilin 2), SIGLEC15 (Sialic Acid Binding Ig Like Lectin 15), GSC (Goosecoid Homeobox), IGFBP1 (Insulin Like Growth Factor Binding Protein 1), LRATD1 (LRAT Domain Containing 1), CXADR (CXADR Ig-Like Cell Adhesion Molecule), CLMP (CXADR Like Membrane Protein), Gad1 (Glutamate Decarboxylase 1), OGT (O-Linked N-Acetylglucosamine (GlcNAc) Transferase), Dazap1 (DAZ Associated Protein 1), Neurod1 (Neuronal Differentiation 1)*

3.1 Association found between epigenetics and prenatal stress

In animal subjects, twenty-six studies disclosed a significant association between epigenetic alterations and fetal stress, three documented an association and merely one showed negative results. While in human studies, seventeen studies revealed a clear significant association between prenatal stress and epigenetics, eight documented a small relation and five showed a negative association.

3.1.1 Human studies.

In a follow-up study, (Cao-Lei et al., 2014) found that aim maternal distress was correlated with DNA methylation levels in 1675 CG sites that along with 957 changes in genes related to immune function. Another study by (Oberlander et al., 2008) found that the methylation status of the *NR3C1* gene in newborns is highly susceptible to prenatal maternal mood shifts. Project ice storm conducted several studies related to prenatal maternal stress where (Cao-Lei et al., 2015) found that prenatal maternal stress was correlated with genes methylated that are involved in type 1 and type 2 diabetes pathways. A birth cohort (Braithwaite, Kundakovic, Ramchandani, Murphy, & Champagne, 2015) discovered that manifestations of prenatal depression led to a significant methylation increase in *NR3C1 1F* gene in male newborns and predicted *BDNF* decreased DNA methylation in female and male infants. A genome-wide association study (Non, Binder, Kubzansky, & Michels, 2014) identified small DNA methylation changes in 42 CpG sites in neonates that were exposed to non-medicated depression or anxiety in contrast to controls. (Vangeel et al., 2015) provided additional evidence that prenatal maternal stress can affect DNA methylation of imprinted genes such as *IGF2* and *GNASXL*. A descriptive cohort (Santos et al., 2021) discovered a correlation between maternal exposure to various psychosocial measures and established several interactions with DNA methylation and discrimination. An epigenome-wide study in Norway (Wikenius et al., 2019) revealed no association between maternal exposure to depression and neonatal DNA methylation levels. A locus-specific DNA methylation study (Perroud et al., 2014) established that post-traumatic stress disorder in pregnant mothers was associated with high methylation levels on the *NR3C1* exon 1 F promoter region. Another descriptive study (Montoya-Williams et al., 2017) noticed a strong association ($P = 0.0027$) between the birth weight of newborns and *IGF2* and *PC2* gene methylation in the mother's blood. One longitudinal study which studied psychosocial stress Found a significant association between AVP methylation and sleep disorders throughout pregnancy and after

pregnancy. A clinical examination (Trump et al., 2016) demonstrated that significant levels of stress throughout pregnancy was associated with an increased risk for recurrent wheezing in children until the age of five, and most methylated areas were associated with Wnt signaling/ calcium pathway in children which is a crucial developmental pathway for lung maturation during gestation. (Vangeel et al., 2017) represented that anxiety throughout pregnancy is associated with distinct DNA methylation patterns in newborns and with *GABBR1* being the most noteworthy as it is associated with infant cortisol levels or HPA axis stress response. Furthermore, (Grasso et al., 2020) demonstrated that trauma-related symptoms in pregnancy showed allele-specific associations of *FKBP5* methylation patterns, however in mothers carrying stress-sensitive CT and TT genotypes, there was a negative correlation between maternal *FKBP5* methylation and maternal PTSD symptoms. In contrast to CC homozygotic infant genotype as it presented a clear correlation between *FKBP5* methylation and maternal PTSD symptoms.One of the most common genes found related to prenatal stress is the insulin/like growth factor 2 (*IGF2/H19)* decreased DNA methylation in newborns after exposure to maternal anxiety particularly in females and neonates with small birth weight. It has been shown that *IGF2/H19* are imprinted candidate genes that function in development and fetal growth and that any methylation changes that occur in this region have been proven to be an instigator of metabolic profile reprogramming in infants (Su et al., 2016). (Vidal et al., 2014) revealed a potential correlation where elevated maternal stress levels were associated with greater infant DNA methylation at the imprinted mesoderm-specific transcript (*MEST*) differentially methylated gene. Loss of imprinting of this gene has been linked to diverse types of cancer as it plays a potential role in development (RefSeq, Dec 2011). Another study (Oh et al., 2013) decided to examine whether the effects of maternal depression have any effects on infant *NR3C1 1-F* promoter DNA methylation at CpG unit 22 and 23 sites and they settled that *NR3C1 1-F* promoter methylation was stronger in newborns who had been exposed to depressed levels of maternal depression in utero. While, (Ostlund et al., 2016) studied the same gene but examined whether DNA methylation of the glucocorticoid receptor gene, *NR3C1*, was associated with experiences of stress by an expectant mother and fearfulness in her newborn and the results suggest an association between prenatal stress and *NR3C1* exon 1F for females but not males which suggest an experience-dependent pathway to fearfulness for female infants. Kallak et al., 2021 examined the association between prenatal depression and DNA methylation and documented no CpG sites were identified relating to prenatal depression alone but there were DNA methylation differences in cord blood of children whose mothers had prenatal depression, anxiety, and selective serotonin reuptake inhibitors (SSRIs). Kertes et al., 2017 The first study to examine BDNF methylation and its association with prenatal maternal stress vulnerability in three tissues at the same time, and the original to report an association of maternal stress and high

BDNF methylation in placental tissue. Findings from (Kertes et al., 2016) which examined the effects of persistent maternal stress and war trauma in HPA axis genes showed that significant methylation was linked with all four genes *CRH, CRHBP*, *NR3C1*, and *FKBP5* with *CRH* being restricted to cord blood and *NR3C1* in the placenta. It is essential to note that *FKBP5* and *CRH* genes have been implicated in great risks of post-traumatic disorder development and peritraumatic dissociation (Jaksic et al., 2019). Also, (Stroud et al., 2016) detected that both placental HSD11B2 methylation and SLC6A4 gene expression controlled the jolt of prenatal major depression disorder on infant cortisol regulation. Findings were affected by neonatal sex, as the strongest *HSD11B2* effects observed females and *SLC6A4* only in males. One study examined the effects between dopamine receptor methylation and prenatal maternal stress on the methylphenidate MPH response in ADHD children and indicated that there was a substantial interaction effect ($p = 0.0001$) of CpG7 of the *DRD4* gene and prenatal stress. A birth cohort demonstrated that during the critical duration of in utero development, *FKBP5* showed increased methylation on five CpGs located on intron 5 (Duis et al., 2018). Infants whose mothers reported depression during pregnancy showed high placental methylation levels in *NR3C1* CpG2 and reported low self-management, hypotonia, and higher fatigue than children whose mothers did not report depression. While DNA methylation in placental *11β-HSD-2* CpG4 infants whose mothers reported anxiety during pregnancy was greater compared to the control group (Conradt, Lester, Appleton, Armstrong, & Marsit, 2013). A prospective study (Rijlaarsdam et al., 2017) examined *OXTR* rs53576 genotype sensitivity for neonatal *OXTR* DNA methylation about prenatal maternal stress exposure and child autistic traits. they discovered that *OXTR* methylation levels were positively associated with social problems for *OXTR* rs53576 G-allele homozygous children but not for A-allele carriers. Another birth cohort revealed that prenatal maternal stress was associated with methylation sites in pathways involved with zinc finger proteins (Letourneau et al., 2021). Additionally, (Unternaehrer et al., 2016) investigated whether maternal stress and cortisol levels throughout pregnancy could be predictive of cord blood DNA methylation of the oxytocin receptor *OXTR* and there was no statistically significant association between maternal prenatal stress, cortisol levels, and *OXTR* DNA methylation. The sole study found in this systematic review narrowed search in human studies investigating noncoding RNA epigenetic modifications in maternal prenatal stress found that methylation status of the miR-30a gene is substantially reduced in the hippocampus and PFC of adult male and female rats whose mothers had been exposed to stress during the last week of gestation (Cattaneo et al., 2020). Maternal distress and salivary cortisol and DNA methylation of three glucocorticoid pathway genes *HSD11B2, NR3C1*, and *FKBP5* in term placentas were examined about fetal movement and heart rate. Results indicated a modest methylation elevation of *HSD11B2* with poor fetal coupling, modest *FKBP5* methylation,

and fetal coupling (Monk et al., 2016). Lastly, the association between maternal emotional state and cortisol levels during pregnancy was assessed with the methylation state of the *NR3C1* promoter region and data revealed that CpG9 was undoubtedly associated with maternal emotional wellbeing (Hompes et al., 2013).

3.1.2 Animal studies

In a study where pregnant mice were exposed to stress during the initial week of pregnancy, it was observed that males were demasculinized behaviourally and physiologically. Gene expression patterns in demasculinized males showed differentially expressed microRNAs in their brains that closely resembled that of control females (Morgan & Bale, 2011). An agricultural study in mature brahman cows examined DNA methylation patterns as mechanisms for altered behavior and stress response in prenatally stressed groups compared to controls and they found 14 genes differentially methylated related to the stress response and behavior in the PNS group (Littlejohn et al., 2019). Similarly, Cilkiz et al., 2020 examined prenatal transportation impact on DNA methylation of lymphocytes of suckling Brahman bull cows in their first five years of life and detected substantial genome-wide variations in lymphocytes of female brahman cattle owing to prenatal stress exposure and aging (Cilkiz et al., 2020). In contrast, rodents are generally utilized in prenatal stress models. Thus, to address the effect of prenatal restraint stress in pregnant rats for *Reelin* gene expression, rats suffered restrain every day for two hours. Significant methylation of the reeling gene promoter in the cortex of prenatally stressed newborn rates was detected, hence these results illustrated the effects of prenatal stress on behavior and physiology (Palacios-García et al., 2015). Another study produced DNA methylation as a potential mechanism through which prenatal stress altered DNA methylation of *Hsd11b2* gene expression between the placenta and fetal cortex which highlighted the tissue specificity of epigenetic effects (Jensen Peña, Monk, & Champagne, 2012).Repeated exposure to restraint stress also seemed to increase *Bdnf* methylation levels in the hippocampus and amygdala of prenatally stressed rats (Boersma et al., 2014). Additionally, the jolt of prenatal environment stress and maternal western diet was also studied and the results indicated changes in miR-10b promoter which led to decreased Bdnf levels in a mouse model of reduced offspring hippocampal neurogenesis and cognition (Ke, Huang, Fu, Lane, & Majnik, 2021)The effect of negative maternal environment was examined on microRNA profile and epigenetic properties in addition to *Bdnf* expression in hippocampal tissue of mice have been studied. Changes in the epigenetic profile of the miR-10b promotor could affect the levels of its metabolites and lower *Bdnf* levels which impairs hippocampal regions of the fetus (2021; Ke, Huang, Fu, Lane, and Majik).The preclinical study (Dong, Tueting,

Matrisciano, Grayson, & Guidotti, 2016) aimed to determine the DNA methylome profile observed in bipolar and schizophrenic patients Accordingly, mice were exposed to repeated episodes of prenatal stress which resulted in increases in 5MC and 5HMC at promoter regions of *Reln* ,*Gad1* and *Bdnf* genes which are correlated with social interaction and locomotor activity. To look at the long-term impact of exercise before pregnancy on prenatally stress-induced alterations (Luft et al., 2020), implemented a prenatal restraint stress model where mice showed fear and anxiety behavior and they noticed that pregestational exercise may reduce developmental modifications that are triggered by prenatal stress in a sex-dependent fashion through regulation of *Crhr1* gene in females and histone 3 acetylation in males. Another study examined the effects of genipin (a chemical compound found in Genipa Americana fruit extract) on the progeny of prenatally stressed mice and discovered that vulnerability to stress during pregnancy decreased expression of dnmt1, hippocampal *Bdnf* due to methylation on promoter sites (Ye, Zhang, Fan, Zhang, & Dong, 2018). Exploring molecular modifications in the premature brain development of mouse embryos exposed to the mother's gene-environment allowed (Sjaarda et al., 2017) to find that the offspring of *Slc6a4* (+/+) dams showed significantly altered methylation profiles, 157 differential expressions of miRNAs, altered expression of 1009 genes involved in neuronal development and cell adhesion pathways. This inadequate response could lead to a stronger risk of developing ASD-like syndromes in offspring of prenatally stressed mothers. Monteleone et al., 2013, analyzed the impact of prenatal stress on *Gmp6a* gene expression an epigenetic mechanism involved, and results show that prenatal stress led to the overexpression of miR-133b which affected *Gmp6a* levels. The *Gmp6a* gene is found in the hippocampus and it functions in neuronal growth, filopodium/spine formation, synapse formation, and filipodium movement (Monteleone et al., 2013). An association study examined the impact of chronic unpredictable prenatal stress on memory and epigenetic measures (histone and DNA methylation) in epigenetic offspring and found that acetylated histone H3 (Lys 14) levels decreased in the dorsal hippocampus due to prenatal stress in both sexes, but more in females and *Dnmt1* levels increased significantly in females only. These findings suggest that prenatal stress could epigenetically alter the hippocampus especially in females (Benoit, Rakic, & Frick, 2015). A genome-wide hippocampal DNA methylation study in 5-Htt × prenatal stress model showed a differentially methylated region in the gene encoding myelin basic protein (*Mbp*) which increased depression-like behavior, especially in female offspring. These results insinuate that the molecular mechanisms behind the behavioral effects are promoter methylation-dependent (Schraut et al., 2014). A locus-specific methylation analysis based study (Blaze et al., 2017) aimed to characterize changes in *Bdnf iv* DNA methylation and telomere length through different adult rat brain regions after unpredictable variable prenatal stress and found that male offspring exhibited higher levels of *Bdnf iv*

methylation in the medial prefrontal cortex (mPFC) in contrast to non-stressed males and stressed females. Another interesting finding from this study is that stressed animals tended to have shorter telomeres than controls in their mPFC (Blaze et al., 2017). Methylation levels of *Pdlim5* promoter showed great gender differences in prenatally stressed offspring rats and offspring showed a significant decrease in sucrose preference and an increase in immobility time. Also, *Pdlim5* is found in hippocampal neurons at their presynaptic nerve endings. The results offer a new mechanism of interest for understanding depression and provide experimental evidence for sex-biased precise treatment (Lu et al., 2020).Another interesting genome-wide study studied placental expression genes in PS mice and hypothesized that the stress response is sex-biased in mice. Findings from this study showed that the hypothalamic gene expression in addition to epigenetic miRNAs in the neonatal brain of placental specific hemizygous Ogt gene was significantly altered (Howerton, Morgan, Fischer, & Bale, 2013). A preclinical study that offers a suitable model for studying behavioral and epigenetic changes in patients without depression investigated the impact of gestational stress and found that prenatally stressed offspring exhibited decreased expression of Bdnf and AcH3K14 in the hippocampus, increased expression of *Dnmt1*, *Hdac1*, and *Hdac2*. (Zheng, Fan, Zhang, & Dong, 2016). Similarly, results from a study that examined if there are any sex differences in the epigenetic profile of PS rats showed that PS increased anxiety-like behavior in females, while depression-like behavior was more common in male offspring. These differences in methylation patterns are more likely due to the role of Dnmt1 and demethylase which could have implications for the management of stress-related disorders (Lei, Wu, Gu, Ji, & Yang, 2020). DNA epigenetic modifications in the basolateral amygdala of pregnant mice are associated with the development of an anxiety-like phenotype. These animals exhibited an anxiety-like phenotype, which was accompanied by an increase in Dnmt1 and a reduction in Gad1 expression (Zhu et al., 2018).One study examined the effects of prenatal restraint stress on anxiety- and depression-related behavior in both male and female adult Sprague-Dawley rats and their epigenetic profiles. During pregnancy, the expression of 948 and 44 genes was affected by prenatal stress. These genes were differentially expressed by PS in male and female offspring. Prenatal stress significantly increases the levels of anxiety and depression-related behavior in male rats, but not in female offspring (Van den Hove et al., 2013). It has been hypothesized that maternal PTSD could delay the development of the offspring's physical and behavioral abilities. The mechanism involved could be a dysregulated whole-genome methylation which affects gene expression and changes in neurotransmitters (Zhang et al., 2016). An animal model shows that prenatal restraint stress (PRS) triggers epigenetic changes in certain genes (*Bdnf*) that are implicated in schizophrenia (SZ). Adult offspring exhibit behavioral abnormalities that are like those seen in patients with SZ. These changes are associated with changes

in the molecular components of the brain (Dong et al., 2015). Research by (Mychasiuk, Ilnytskyy, Kovalchuk, Kolb, & Gibb, 2011) revealed that prenatal stress can affect long-term brain development where mild prenatal stress increased global DNA methylation levels in the brain's frontal cortex and hippocampus and high prenatal stress showed a great decrease. High prenatal stress slowed the development of sensorimotor skills and decreased locomotion, and the effects of prenatal stress on the developing brain were different for both males and females.

Another study revealed that prenatal stress is linked to the demethylation of the promoter of the *Crh* gene, which leads to higher levels of anxiety and hyperresponsiveness in adolescent life stages (Xu, Sun, Gao, Cai, & Shi, 2014). The demethylation of CpG-dinucleotides in the promoter of the Crh protein was observed in rats prenatally stressed. These results suggest that the stress-induced by pregnancy alters the normal HPA function of the promoter. Another animal study (Mueller & Bale, 2008), found that male offspring exposed to stress early in gestation displayed maladaptive behavioral stress responsivity, anhedonia, and increased sensitivity to selective serotonin reuptake inhibitor treatment. Long-term alterations in central *Crf* and GR expression, as well as increased HPA axis responsivity, were present in these mice and likely contributed to elevated stress sensitivity.

3.2 Epigenetic mechanisms associated with prenatal stress

As for the epigenetic mechanisms underlying prenatal stress studied in humans, twenty-nine studies examined DNA methylation (locus specific: 18 and genome wide association study: 8) and only one study focused on noncoding RNAs. In contrast, the epigenetic mechanisms studies in animals included, eighteen studies on DNA methylation (locus specific: 11 and genome wide: 7), four on histone modifications (locus specific: 2 and genome wide: 2) and eight on noncoding RNA mainly miRNAs (locus specific: 2 and genome wide: 7).

3.3 Epigenetic profiling methods used in maternal prenatal stress

The methods used in DNA methylation and noncoding RNA epigenetic profiling in human studies were a) bisulfite pyrosequencing in eleven studies, PCR in two studies, and Microarrays in 7 studies, and Infinium methylation 450 Bead chip arrays in ten studies. Methods used animal studies were: a) bisulfite pyrosequencing in 6 studies, b) PCR in thirteen studies (qRT- PCR: 4, RT-PCR: 5, qPCR : 2, and conventional PCR), c) ChIP was used in four studies, and d) MeDIP in seven studies.

3.3.1 Human studies

Table 3 Methods used according to the type of study, epigenetic modification and tissue type.

Author	Type of study & Epigenetic modification	Method (s) used	Tissue type
(Cao-Lei et al., 2014)	Follow up comparative study, DNA methylation	Infinium Human Methylation 450 BeadChip Array, Bisulfite treatment and pyrosequencing	blood cells and saliva samples
(Oberlander et al., 2008)	Association study, locus specific DNA methylation	bisulfite pyrosequencing.	cord blood and salivary cortisol
(Cao-Lei et al., 2015)	longitudinal study, Global DNA methylation	Infinium Human Methylation 450 BeadChip Array,	blood for T cell isolation
(Braithwaite, Kundakovic, Ramchandani, Murphy, & Champagne, 2015)	Birth Cohort, locus specific DNA methylation study	quantitative bisulfite-pyrosequencing method using PyroMark Q24 pyrosequencer	salivary cortisol and buccal swabs (from infants- 2 months age)
(Non, Binder, Kubzansky, & Michels, 2014)	GWAS, DNA methylation study	Illumina Infinium Human Methylation450 BeadChip	umbilical cord blood of neonates
(Vangeel et al., 2015)	Birth cohort, Locus specific DNA methylation study	Sequenom EpiTYPER.	cord blood
(Santos et al., 2021)	Descriptive cohort, Locus specific DNA methylation analysis	bisulphite pyrosequencing.	Blood samples
(Wikenius et al., 2019)	Longitudinal epigenome-wide study, DNA methylation	Illumina Infinium HumanMethylation 450 BeadChip.	saliva cells,
(Perroud et al., 2014)	longitudinal study, Locus DNA methylation analysis	bisulfite conversion, pyrosequencing	peripheral blood leukocytes
(Montoya-Williams et al., 2017)	Descriptive cohort, locus specific DNA methylation	Illumina Human methylation 450 Bead Chip	Cord blood
(Solomonova et al., 2019)	Longitudinal study, DNA methylation	vasopressin methylation analysis	Saliva samples
(Trump et al., 2016)	Clinical Trial & DNA methylation	Whole genome bisulfite sequencing (WGBS), Validation analysis: MassARRAY and qPCR	whole blood samples
(Vangeel et al., 2017)	Cohort, DNA methylation	HumanMethylation450 BeadChip and EpiTYPER	Cord blood
(Grasso et al., 2020)	Association study, DNA methylation	Bisulfite pyrosequencing and DNA methylation analysis	Saliva from postpartum infants
(Mansell et al., 2016)	cohort study, DNA methylation	Sequenom MassArray Platform	cord blood mononuclear cells
(Vidal et al., 2014)	Genome wide association study, DNA methylation	bisulfite pyrosequencing	Peripheral cord blood
(Oh et al., 2013)	Descriptive study, locus specific DNA methylation	Sequenom EpiTYPER system	saliva and cord blood

(Ostlund et al., 2016)	Comparative study, Locus specific DNA methylation	Bisulfite Pyrosequencing DNA Methylation Analysis	Buccal cells from each infant
(Kallak et al., 2021)	DNA methylation, Association study	Bisulfte conversion of DNA	Cord blood
(Kertes et al., 2017)	Cohort, Locus specific DNA methylation	Epigenotyping using Illumina HumanMethylation450 BeadChips and Sodium bisulfate sequencing	umbilical cord blood, placental tissue, and maternal venous blood
(Kertes et al., 2016)	Association study, DNA methylation, locus specific	Illumina HumanMethylation450 BeadChips	neonatal cord blood, placenta, and maternal blood
(Stroud et al., 2016)	Descriptive study, Locus specific DNA methylation	bisulfite Pyrosequencing System (Qiagen)	Placenta samples
Kim, Kim, Shin, & Kim, 2018)	Prospective study, Locus specific DNA methylation	bisulfite sequencing	whole blood
(Duis et al., 2018)	Birth cohort, Locus specific DNA methylation analysis	Bisulfite Pyrosequencing	cord blood samples,
(Conradt, Lester, Appleton, Armstrong, & Marsit, 2013)	Birth Cohort, Locus specific DNA methylation	Bisulfite pyrosequencing DNA methylation analysis	Placenta sample collection, nucleic acid extraction, and bisulfite modification
(Rijlaarsdam et al., 2017)	Prospective study, Locus specific DNA methylation	Illumina Infinium HumanMethylation450 BeadChips,	cord blood samples,
(Letourneau et al., 2021)	Birth Cohort, Locus DNA methylation study	IlluminaHumanMethylation450 array	3-month-old blood samples
(Unternaehrer et al., 2016)	Cross-sectional study, Locus specific DNA methylation	OXTR DNA methylation was quantified using Sequenom EpiTYPER	cord blood, maternal saliva samples,
(Cattaneo et al., 2020)	Comparative study, miRNA-30a	Methylated DNA immunoprecipitation (MeDIP) analysis and Gene and miRNA expression analyses by Real Time PCR	Hippocampal tissue and prefrontal cortex and peripheral blood samples for miRNA-30 analysis from humans
(Monk et al., 2016)	Prospective study, DNA methylation locus specific	Placental CpG methylation in the three genes was analysed using 450K Beadchips and bisulfite sequencing	salivary cortisol and placental tissue
(Hompes et al., 2013)	Association study, locus specific DNA methylation	. Bisulfite conversion and Sequenom Epityper MassARRAY	Cord blood mononuclear cells

3.3.2 Animal studies

Table 4 Methods used according to the species, type of study, epigenetic modification and tissue type.

Author	Species	Type of study & Epigenetic modification	Method (s) used	Tissue type
(Morgan & Bale, 2011)	Mouse C57BL/6	Comparative study & miRNAs	Custom Taqman qRT-PCR Array, Tail suspension test.	Whole brains
(Littlejohn et al., 2019)	Mature Brahman cows	Comparative Study, DNA methylation	bisulfite sequencing methods, Methyl-MiniSeq	white blood cells
(Palacios-García et al., 2015)	Rats	Comparison study, DNA methylation	western blot and DNA methylation assay - Conventional PCR and behavioral tests	Adrenal glands, E20 fetal brains (frontal, parietal and retroslpenial brain sections)
(Jensen Peña, Monk, & Champagne, 2012)	Long-Evans Rats	Behavioural study, DNA methylation	Purified DNA was analyzed (EpigenDX) for CpG methylation by bisulfite pyrosequencing	placenta, fetal brains (cortex, hypothalamus)
(Cilkiz et al., 2020)	Brahman heifer calves,	Longitudinal study, DNA methylation	reduced representation bisulphite sequencing	whole blood cells
(Zucchi et al., 2013)	Rats	Comparative study, non-coding RNA (miRNA)	Quantitative real time PCR and gene microarray expression analysis	brain tissue (frontal cortex-mothers) and entire brains of male newborn offspring
(Boersma et al., 2014)	Rats	Association locus specific DNA methylation study	Bisulfite pyrosequencing Genomic DNA	Prefrontal cortex (PFC), and hippocampus (HPC) tissue
(Ke, Huang, Fu, Lane, & Majnik, 2021)	Mouse C57/Bl6	Association study, non-coding RNA (miRNA)	RT-PCR, pyrosequencing and CHIP Chromatin Immunoprecipitation Assay	Hippocampal tissue
(Dong, Tueting, Matrisciano, Grayson, & Guidotti, 2016)	Mouse	Comparison study, DNA methylation	Methylated DNA immunoprecipitation using MeDIP, ChIP assays	Frontal cortex samples
(Luft et al., 2020)	Mouse	Comparative study, Histone modifications	EpiQuiK Global Histone H3 Acetylation Assay Kit	Prefrontal cortex tissue was used for corticosterone, gene expression, and epigenetic analysis - and blood samples

(Ye, Zhang, Fan, Zhang, & Dong, 2018)	Mouse model, kumming	Preclinical study, DNA methylation	methylated DNA immunoprecipitation and qPCR	Hippocampal tissue
(Sjaarda et al., 2017)	Mouse	Scientific report, Genome wide DNA methylation	Whole genome profiling of methylome, transcriptome and miRNA using Next Generation Sequencing	post stress embryonic brains, tissue from 3 placentas and 3 embryos for each experimental maternal condition
(Monteleone et al., 2013)	Wistar rats	Locus specific DNA methylation and miRNA	retrotranscription and qPCR to identify miRNA levels and, bisulfite conversion,	Hippocampus and prefrontal cortex samples
(Benoit, Rakic, & Frick, 2015)	Mouse	Association study, both locus DNA methylation & Histone modification	Measure levels of acetylated H3 at lysine-14 (AcH3Lys14) using western blotting	blood and dorsal hippocampus (because it's involved in spatial memory)
(Schraut et al., 2014)	Mouse , C57BL6/J	Locus specific methylation comparison study, DNA methylation	Genome-wide hippocampal DNA methylation screening using methylated-DNA immunoprecipitation (MeDIP) on Affymetrix GeneChip Mouse Promoter 1.0 R arrays	Hippocampal tissue
(Blaze et al., 2017)	Rats	Association study, locus DNA methylation analysis	DNA was bisulfite-converted and direct bisulfite-sequencing PCR (BSP) was used to measure methylation	mPFC, ventral hippocampus, dorsal hippocampus, and central/basolateral amygdala
(Lu et al., 2020)	Rats	Locus specific methylation analysis &DNA methylation	qRT-PCR and western blot, and bisulfite sequencing	Hippocampal tissue
(Howerton, Morgan, Fischer, & Bale, 2013)	Mouse	Genome wide association study, miRNA modification	Taqman Array MicroRNA card A Array (Applied Biosystems).	embryonic somatic tissue, brain tissue, and human placental tissues
(Zheng, Fan, Zhang, & Dong, 2016)	Mouse	DNA methylation and histone modifications	RT-qPCR, Western blot , ChIP, methylated DNA immunoprecipitation (MeDIP)	10mg of Hippocampal tissue
(Lei, Wu, Gu, Ji, & Yang, 2020)	Rats	Preclinical study. Locus specific methylation	pyrosequencing, western blotting, and Golgi staining to assess changes in methylation	hippocampal tissue samples
(Zhu et al., 2018)	Mouse	Preclinical study, DNA methylation and Histone modifications	. Real-time RT-PCR, western blot, chromatin immunoprecipitation, and electrophysiological analysis	entire brains and basolateral amygdala
(Van den Hove et al., 2013)	Rats	Preclinical study, Histone modification	Microarray-based profiling	Hippocampus and frontal cortex

(Zhang et al., 2016)	Rats	Genome wide association study, DNA methylation	gene expression profile chip tests, and methylated DNA immunoprecipitation sequencing (MeDIP-Seq)	blood samples
(Dong et al., 2015)	Mouse	Preclinical study, Histone and DNA methylation	MeDIP, hMeDIP kits ,and chromatin immunoprecipitation (ChIP) assays .	frontal cortex and hippocampus.
(Mychasiuk, Ilnytskyy, Kovalchuk, Kolb, & Gibb, 2011)	Rats	Genome wide association study, DNA methylation	qRT-PCR	The frontal cortex and hippocampus
(Cattaneo et al., 2020)	Rats and Human study	Comparative study, miRNA-30a	Methylated DNA immunoprecipitation (MeDIP) analysis and Gene and miRNA expression analyses by Real Time PCR	Hippocampal tissue and prefrontal cortex and peripheral blood samples for miRNA-30 analysis from humans
(Niknazar et al., 2016)	Rats	Compartive study, DNA methylation locus specific	real-time PCR and Western blotting in all groups	entire hippocampus
(Mueller & Bale, 2008)	Mouse	behavioral study, GWA DNA methylation	Pyrosequencing was performed by EpigenDx, TaqMan Gene Expression Assay	Adult brain tissue collection from males exposed to prenatal stress and placental tissue
(Xu, Sun, Gao, Cai, & Shi, 2014)	Spragye-Dawley rats	Locus specific DNA methylation study	Genomic DNA Extraction and Bisulfite Sequencing	hypothalamus

3.4 Genes, miRNAs, and histone proteins in Prenatal maternal stress

The most common genes associated with maternal prenatal stress found in human studies were: *NR3C1* in eight studies, *FKBP5* in five studies, *IGF2* in three studies, *BDNF* in two, *SLC6A4* in two, *HSD11B2* in two studies, *LTA* in two, *PC2* in two studies, and *CRH* in two studies. In contrast to animal studies where in animal studies the common genes found were: *BDNF* in eight studies, *DNMT* in eight, H3K4 in four, *GAD1* in two, *TET* in two, Reelin in two studies.

Table (5) Protein coding genes and their relevance in the HPA axis response.

Gene	Protein coding	Functional relevance to HPA axis
NR3C1 Nuclear Receptor Subfamily 3 Group C Member 1	This gene encodes glucocorticoid receptors, which bind to glucocorticoids response elements of glucocorticoid responsive genes to activate or repress their transcription.	*NR3C1* plays a central role in the stress response; as glucocorticoids (i.e., cortisol) are released through activation of the HPA axis they bind to glucocorticoid receptors, which serve to inhibit further HPA axis activation.
11β-HSD2 Hydroxysteroid 11-Beta Dehydrogenase 2	This gene encodes a type II enzyme that catalyzes cortisol into cortisone.	*11β-HSD2* is expressed in the placenta and serves to protect the fetus from excess cortisol exposure, which can lead to dysregulation of the fetal HPA axis.
FKBP5 FK506 Binding Protein 5	This gene encodes for a protein relevant for immunoregulation and other cellular processes requiring protein folding and trafficking.	*FKBP5* aids in HPA axis regulation by terminating the stress response through determining sensitivity to glucocorticoid receptors.
CRH Corticotropin Releasing Hormone	This gene encodes for a protein that aids in the generation of the peptide hormone, corticotropin.	*CRH* aids in synthesizing adrenocorticotropic hormone (ACTH), which stimulates the production and release of cortisol by the pituitary gland, as part of the HPA axis.
CRHBP Corticotropin Releasing Hormone Binding Protein	This gene encodes for a protein that aids in the synthesis of CRH.	*CRHBP* regulates the availability of CRH at its relevant receptors.
SLC6A4 Solute Carrier Family 6, Member 4	This gene encodes for a protein that is integral in the transmission of serotonin from synaptic spaces into presynaptic neurons.	Decreased production of serotonin via *SLC6A4* has been implicated in alterations of serotonergic modulation of the HPA axis (Fuller, 1990).
OXTR Oxytocin Receptor	This gene encodes for a protein that acts as a receptor for oxytocin.	*OXTR* has been implicated in the regulation of the HPA axis in humans by suppressing the production of cortisol and ACTH (Heinrichs & Domes, 2008).

Image extracted from https://pubmed.ncbi.nlm.nih.gov/29344930/ (Sosnowska et al., 2018).

4. Discussion

The present systematic review resulted in multiple findings for the association between maternal prenatal stress, DNA methylation, histone modifications, non-coding RNA changes, methods used, and genes found. The primary finding of this systematic review is that the prenatal period is a critical time for neonatal development and that maternal prenatal stress could regulate the epigenome of the fetus through epigenetic modifications. The aim of this study is to examine the effects of epigenetics in maternal prenatal stress. The exclusive design of the review, which included DNA methylation, histone modification, and noncoding RNA studies, yielded more information that can strengthen interpretations of the findings. One is the understanding of factors that can cause null findings, such as effect size, sample size, and method limitations. Another advantage of this review was the holistic search that was done to identify which genes were associated with prenatal stress and what kind of epigenetic modifications are most common. Also, narrowing down the search to psychological stressors instead of environmental factors can help yield a more clear and focused understanding of what are the associations between maternal mental health state and the effects on offspring. The most frequently studied stressors reported in this review in animal studied comprised restraint stress with slight variations in durations and cycles in each study such as variable stress, chronic variable stress, fear /anxiety behavior, and chronic unpredictable stress model with light cycle disruptions. While in human studies, this review focused on psychological or psychosocial stressors such as major

depression disorder (MDD), depression-like symptoms, anxiety/anxiety-like symptoms, and post-traumatic stress disorder PTSD (Ruiz and Fullerton, 1999).

Given the limited search approach, multiple findings were evident. First, most studies found a modest association between prenatal maternal stress and epigenetics which suggests that prolonged exposure to maternal prenatal stress can most likely affect the transcriptome of the offspring possibly through epigenetic mechanisms (Beydoun and Saftlas, 2008, Eriksson, 2010). However, more studies are needed to assess the behavioral phenotype. The exact mechanism through which maternal prenatal stress is still not yet fully understood since many factors come into interplay. However, one link that can be explained between maternal and fetal cortisol has been suggested as a possible explanation for the effects of antenatal maternal stress on the fetus (Glover, 2015). It is hypothesized that cortisol can cross the placenta and that the placental enzyme *11β-HSD2* exhibits protective effects to the fetus through conversion of cortisol to cortisone before crossing the placenta which leads to the formation of cortisol gradient between the mother and the fetus. Therefore, some cortisol still manages to cross to the fetus, thus elevating fetal glucocorticoid levels (Seckl and Holmes, 2007). Second, that DNA methylation is the most studied epigenetic modification in human and animal studies while histone modifications and non-coding RNA are less studied. However, multiple studies were found highlighting the importance of the miRNA-30 family and their role in mediating the effect of chronic stress on hippocampal neurogenesis in mice (Khandelwal et al., 2019).Similarly, miR-132 was commonly reported in multiple studies and has been shown to play a role in the regulation of neural activity and neuroplasticity (Zheng et al., 2013), and low hippocampus synaptic plasticity in depression and stress (Pittenger and Duman, 2008). Also, miR-10b promotor could influence overexpression levels of miR-10b-5-p which lowers Bdnf levels in prenatally stressed mice and leads to hampered hippocampal neurogenesis and altered cognition (2021; Ke, Huang, Fu, Lane, and Majnik).Third, genome-wide studies were more common in human studies than in animals and the most widely used method used is Infinium HumanMethylation450 Bead Chip since it is cost-effective and covers over 450,000 methylation sites per sample, has high specificity and sensitivity. While locus-specific methods used mainly PCR methods using bisulfite sequencing and pyrosequencing because it determines T/C intensity ratio which leads to accurate analysis of short DNA stretches less than 150 base pairs. Fourth, is that *NR3C1* and *FKBP5* (Grasso et al., 2020) genes which are part of the glucocorticoid pathway were the most studied in human studies where they showed the strongest association between prenatal stress and epigenetics (Perroud et al., 2014, Braithwaite, Kundakovic, Ramchandani, Murphy, & Champagne, 2015, Oberlander et al., 2008, Perroud et al., 2014, Oh et al., 2013, Ostlund et al., 2016, Conradt, Lester, Appleton, Armstrong, &

Marsit, 2013, Hompes et al., 2013). *BDNF* and *DNMT1* were the most studied in animal studies and showed a strong association. BDNF is a growth factor that is involved in neurodevelopment and has been considered as an epigenetic marker of early life stress when its expression is altered (Korsching, 1993, Whitaker-Azmitia et al., 1996). Some genes that were less studied provided some interesting insights, for example, lymphotoxin alpha (*LTA*) gene which is part of the tumor necrosis factor family gene, and Neuroendocrine protein 7B2 (*SCG5*- chaperone protein for *PC2*), proprotein convertase *PC2* which function in regulating the innate and adaptive immune system are both highly associated with aim maternal prenatal stress (Zhang et al., 2016, Cao-Lei et al., 2014). Henceforth, the interactions between genes from different biological pathways could help explain the complexity of the process of epigenetic regulation. It is also possible that prenatal stress could lead to epigenetic genome-wide changes (Cao-Lei et al., 2015b, Cao-Lei et al., 2014). Most of the methylated genes play a part in a specific pathway and only represent a small part of the mechanism, for example, the *FKBP5* gene plays a significant in ovarian steroidogenesis. It's rather difficult to determine whether the methylated genes result from causation or a consequence of other confounding factors. When investigating the developmental properties of multicellular organisms, reductionism seems to have its shortcomings (Fang & Casadevall, 2011, Mazzocchi, 2008, Regenmortel, 2004). To detect the associated phenotype from a genotype needs more than the parts. An understanding of the interactions during different parts of development is crucial (Perrimon et al., 2012). While causation cannot be established, it was common to detect that some of these genes and others were differentially expressed in a sex-dependent manner in stressed male and female offspring as reported by seven studies found in this systematic review (Mychasiuk, Ilnytskyy, Kovalchuk, Kolb, & Gibb, 2011) (Van den Hove et al., 2013) (Lei, Wu, Gu, Ji, & Yang, 2020) (Lu et al., 2020) (Blaze et al., 2017) (Benoit, Rakic, & Frick, 2015) (Ye, Zhang, Fan, Zhang, & Dong, 2018). This is not surprising since the brain exhibits a bipotential characteristic and develops under the impact of sex-specific steroid hormones and genetic factors in sex chromosomes in females and males throughout the prenatal critical period (Corbier et al., 1992). It's also crucial to note that in rodents, brain organization occurs postnatally from P0 to P10, while in humans it occurs throughout the second trimester McCarthy et al., 2009). Animal studies highlighted the importance of the hippocampus as a crucial area in the HPA stress response, where chronic stress is accompanied by a decrease of the glucocorticoid receptor in the hippocampus (Kitraki et al., 1999). Studies on maternal stress reveal that it can change the development of the fetus and increase the risk of various disorders in adulthood (Charil et al., 2010, Glover, 2011). The timing of stress exposure during pregnancy is also a significant factor in epigenetic studies (Veru et al., 2014a). It has been shown that maternal stress at

the early stages is associated with epigenetic changes that are more significant than those at later stages of pregnancy (Hompes et al., 2013, Mueller and Bale, 2008, Oberlander et al., 2008b).

In conclusion, exposure to maternal prenatal stress could cause epigenetic changes in neonates in both animals and humans. In this systematic review, locus-specific and genome-wide association studies in humans and animals studying maternal prenatal stress along with the epigenetic modifications were outlined and summarized. This paper also briefly discussed the current trends and methods being used in epigenetic profiling and genes that are commonly associated with prenatal stress.

4.1 Limitations

4.1.1 Limitations within the literature

Although the current review provided useful findings, it should be noted that future studies should focus on the role of other epigenetic modifications besides DNA methylation in the development of infants. The first is that the gestation cycle of mice is short in contrast to humans doesn't accurately model prenatal stress. Second, is that studies on genes other than *NR3C1* should be performed in the infant epigenome since it will expand our understanding of maternal prenatal stress and the epigenetic mechanisms involved. Third, the lack of specificity of prenatal stress timing occurrence has prevented many studies from estimating when prenatal stress took place. Fourth, the lack of assessments of relevant covariates is a limitation of the current literature. Studies that exclude participants who do not have covariates can exclude key findings and biases. Finally, most studies conducted on DNA methylation only drew data from saliva and peripheral blood samples, which may limit the generalizability and usefulness of their findings (Benvenisty et al., 1985). Also, the sample size was an issue across studies, which could make it hard to detect all potential effects. This limitation should not be ignored in studies related to DNA methylation It should also be noted that the developmental effects of DNA methylation on humans are not yet fully understood.

4.1.2 Limitations within this review

This review has several limitations. The first limitation of this study is the small sample of literature found which yielded 59 articles this was because of limiting the search procedure to one database PUBMED/MEDLINE and excluding environmental stressors in epigenetics which is studied extensively compared to maternal prenatal stress. Second, the replication of this study is needed since the method and the search terms could have included possible study selection bias. Third, the review is limited to genes involved in the HPA axis mainly in the glucocorticoid pathway, serotonergic pathway, and some genes in the immune system. This is important to consider since the HPA axis involves the gut microbiota and the immune system, whose genes could have yielded a more comprehensive understanding of the underlying mechanisms of stress. Fourth, the genes discussed were the ones that are most found more than once in the studies. Finally, it is possible that the search procedure could have inadvertently selected studies that did not discuss the link between maternal stress and epigenetics.

References

Aguilera, O., Fernández, A. F., Muñoz, A., & Fraga, M. F. (2010). Epigenetics and environment: a complex relationship. Journal of Applied Physiology, 109(1), 243–251. doi: 10.1152/japplphysiol.00068.2010

Alyamani, R. A. S., & Murgatroyd, C. (2018). Epigenetic programming by early-life stress. *Progress in molecular biology and translational science*, *157*, 133-150.

Ambrosi, C., Manzo, M., & Baubec, T. (2017). Dynamics and Context-Dependent Roles of DNA Methylation. *Journal Of Molecular Biology*, *429*(10), 1459-1475. doi: 10.1016/j.jmb.2017.02.008

Babicola, L., Ventura, R., D'Addario, S., Ielpo, D., Andolina, D., & Di Segni, M. (2021). Long term effects of early life stress on HPA circuit in rodent models. *Molecular And Cellular Endocrinology*, *521*, 111125. doi: 10.1016/j.mce.2020.111125

Banerjee, S. B., Arterbery, A. S., Fergus, D. J., & Adkins-Regan, E. (2011). *Deprivation of maternal care has long-lasting consequences for the hypothalamic-pituitary-adrenal axis of zebra finches. Proceedings of the Royal Society B: Biological Sciences, 279(1729), 759–766.* doi:10.1098/rspb.2011.1265
Bannister, A., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research*, *21*(3), 381-395. doi: 10.1038/cr.2011.22

Benoit, J., Rakic, P., & Frick, K. (2015). Prenatal stress induces spatial memory deficits and epigenetic changes in the hippocampus indicative of heterochromatin formation and reduced gene expression. Behavioural Brain Research, 281, 1-8. doi: 10.1016/j.bbr.2014.12.001

Bertrand-Lehouillier, V., Legault, L., & McGraw, S. (2019). Endocrine Epigenetics, Epigenetic Profiling and Biomarker Identification. Encyclopedia Of Endocrine Diseases, 31-35. doi: 10.1016/b978-0-12-801238-3.65830-0

Blaze, J., Asok, A., Borrelli, K., Tulbert, C., Bollinger, J., Ronca, A., & Roth, T. (2017). Intrauterine exposure to maternal stress alters Bdnf IV DNA methylation and telomere length in the brain of adult rat offspring. International Journal Of Developmental Neuroscience, 62(1), 56-62. doi: 10.1016/j.ijdevneu.2017.03.007

Boersma, G., Lee, R., Cordner, Z., Ewald, E., Purcell, R., Moghadam, A., & Tamashiro, K. (2013). Prenatal stress decreasesBdnfexpression and increases methylation ofBdnfexon IV in rats. Epigenetics, 9(3), 437-447. doi: 10.4161/epi.27558

Braithwaite, E., Kundakovic, M., Ramchandani, P., Murphy, S., & Champagne, F. (2015). Maternal prenatal depressive symptoms predict infant NR3C11F and BDNF IV DNA methylation. Epigenetics, 10(5), 408-417. doi: 10.1080/15592294.2015.1039221

Bredy, T. W., Wu, H., Crego, C., Zellhoefer, J., Sun, Y. E., & Barad, M. (2007). Histone modifications around inspanidual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *14*(4), 268–276. https://doi.org/10.1101/lm.500907

Bronson, S. L., & Bale, T. L. (2016). The Placenta as a Mediator of Stress Effects on Neurodevelopmental Reprogramming. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 41(1), 207–218. https://doi.org/10.1038/npp.2015.231

Cao-Lei, L., Massart, R., Suderman, M., Machnes, Z., Elgbeili, G., & Laplante, D. et al. (2014). DNA Methylation Signatures Triggered by Prenatal Maternal Stress Exposure to a Natural Disaster: Project Ice Storm. Plos ONE, 9(9), e107653. doi: 10.1371/journal.pone.0107653

Cao-Lei, L., Dancause, K., Elgbeili, G., Massart, R., Szyf, M., & Liu, A. et al. (2015). DNA methylation mediates the impact of exposure to prenatal maternal stress on BMI and central adiposity in children at age 13½ years: Project Ice Storm. Epigenetics, 10(8), 749-761. doi: 10.1080/15592294.2015.1063771

Cao-Lei, L., de Rooij, S. R., King, S., Matthews, S. G., Metz, G. A. S., Roseboom, T. J., & Szyf, M. (2020). Prenatal stress and epigenetics - PubMed. Neuroscience and Biobehavioral Reviews, 117. https://doi.org/10.1016/j.neubiorev.2017.05.016

Cattaneo, A., Suderman, M., Cattane, N., Mazzelli, M., Begni, V., & Maj, C. et al. (2020). Long-term effects of stress early in life on microRNA-30a and its network: Preventive effects of lurasidone and potential implications for depression vulnerability. Neurobiology Of Stress, 13, 100271. doi: 10.1016/j.ynstr.2020.100271

Cilkiz, K., Baker, E., Riggs, P., Littlejohn, B., Long, C., & Welsh, T. et al. (2020). Genome-wide DNA methylation alteration in prenatally stressed Brahman heifer calves with the advancement of age. Epigenetics, 16(5), 519-536. doi: 10.1080/15592294.2020.1805694

Conradt, E., Lester, B., Appleton, A., Armstrong, D., & Marsit, C. (2013). The roles of DNA methylation of NR3C1 and11β-HSD2 and exposure to maternal mood disorder in utero on newborn neurobehavior. Epigenetics, 8(12), 1321-1329. doi: 10.4161/epi.26634

Costantini, D., Metcalfe, N., & Monaghan, P. (2010). Ecological processes in a hormetic framework. Ecology Letters, 13(11), 1435-1447. doi: 10.1111/j.1461-0248.2010.01531.x

Coussons-Read M. E. (2013). Effects of prenatal stress on pregnancy and human development: mechanisms and pathways. Obstetric medicine, 6(2), 52–57. https://doi.org/10.1177/1753495X12473751

Deangelis, J. T., Farrington, W. J., & Tollefsbol, T. O. (2008). An Overview of Epigenetic Assays. Molecular Biotechnology, 38(2), 179–183. https://doi.org/10.1007/s12033-007-9010-y

Denis, H., Ndlovu, '., & Fuks, F. (2011). Regulation of mammalian DNA methyltransferases: a route to new mechanisms. EMBO Reports, 12(7), 647-656. doi: 10.1038/embor.2011.110

Dion, M., Altschuler, S., Wu, L., & Rando, O. (2005). Genomic characterization reveals a simple histone H4 acetylation code. Proceedings Of The National Academy Of Sciences, 102(15), 5501-5506. doi: 10.1073/pnas.0500136102

Dong, E., Dzitoyeva, S., Matrisciano, F., Tueting, P., Grayson, D., & Guidotti, A. (2015). Brain-Derived Neurotrophic Factor Epigenetic Modifications Associated with Schizophrenia-like Phenotype Induced by Prenatal Stress in Mice. Biological Psychiatry, 77(6), 589-596. doi: 10.1016/j.biopsych.2014.08.012

Dong, E., Tueting, P., Matrisciano, F., Grayson, D., & Guidotti, A. (2016). Behavioral and molecular neuroepigenetic alterations in prenatally stressed mice: relevance for the study of chromatin remodeling properties of antipsychotic drugs. Translational Psychiatry, 6(1), e711-e711. doi: 10.1038/tp.2015.191

Dranovsky, A. et al. Experience dictates stem cell fate in the adult hippocampus. Neuron70, 908–923, https://doi.org/10.1016/j.neuron.2011.05.022 (2011).

Duis, J., Cox, O., Ji, Y., Seifuddin, F., Lee, R., & Wang, X. (2018). Effect of Genotype and Maternal Affective Disorder on Intronic Methylation of FK506 Binding Protein 5 in Cord Blood DNA. Frontiers In Genetics, 9. doi: 10.3389/fgene.2018.00648

Dwyer, J. B., & Ross, D. A. (2017). The Nature of Nurture: How Developmental Experiences Program Adult Stress Circuitry. Biological Psychiatry, 81(8), e57–e59. https://doi.org/10.1016/j.biopsych.2017.02.004

Eckhardt, F., Lewin, J., Cortese, R., Rakyan, V., Attwood, J., & Burger, M. et al. (2006). DNA methylation profiling of human chromosomes 6, 20 and 22. Nature Genetics, 38(12), 1378-1385. doi: 10.1038/ng1909

Fang, F. C., & Casadevall, A. (2011). Reductionistic and holistic science. Infection and Immunity, 79(4), 1401–1404. https://doi.org/10.1128/IAI.01343-10

Glasper, E. R., Morton, J. C., & Gould, E. (2010). Environmental Influences on Adult Neurogenesis. Encyclopedia of Behavioral Neuroscience, 485–492. https://doi.org/10.1016/b978-0-08-045396-5.00241-4

Govindaraj, S., Shanmuganathan, A., & Rajan, R. (2017). Maternal psychological stress-induced developmental disability, neonatal mortality and stillbirth in the offspring of Wistar albino rats. PLOS ONE, 12(2), e0171089. doi: 10.1371/journal.pone.0171089

Grasso, D., Drury, S., Briggs-Gowan, M., Johnson, A., Ford, J., & Lapidus, G. et al. (2020). Adverse childhood experiences, posttraumatic stress, and FKBP5 methylation patterns in postpartum women and their newborn infants. Psychoneuroendocrinology, 114, 104604. doi: 10.1016/j.psyneuen.2020.104604

Guzeloglu-Kayisli, O., Semerci, N., Guo, X., Larsen, K., Ozmen, A., & Arlier, S. et al. (2021). Decidual cell FKBP51–progesterone receptor binding mediates maternal stress–induced preterm birth. Proceedings Of The National Academy Of Sciences, 118(11), e2010282118. doi: 10.1073/pnas.2010282118

Hamilton, G., & Rhodes, J. (2015). Exercise Regulation of Cognitive Function and Neuroplasticity in the Healthy and Diseased Brain. Progress In Molecular Biology And Translational Science, 381-406. doi: 10.1016/bs.pmbts.2015.07.004

Hattori, N., & Ushijima, T. (2011). Analysis of Gene-specific DNA Methylation. Handbook of Epigenetics, 125–134. https://doi.org/10.1016/b978-0-12-375709-8.00008-3

Hodge, R. D. et al. Intermediate progenitors in adult hippocampal neurogenesis: Tbr2 expression and coordinate regulation of neuronal output. J Neurosci28, 3707–3717, https://doi.org/10.1523/jneurosci.4280-07.2008 (2008)

Hompes, T., Izzi, B., Gellens, E., Morreels, M., Fieuws, S., & Pexsters, A. et al. (2013). Investigating the influence of maternal cortisol and emotional state during pregnancy on the DNA methylation status of the glucocorticoid receptor gene (NR3C1) promoter region in cord blood. Journal Of Psychiatric Research, 47(7), 880-891. doi: 10.1016/j.jpsychires.2013.03.009

Howerton, C., Morgan, C., Fischer, D., & Bale, T. (2013). O-GlcNAc transferase (OGT) as a placental biomarker of maternal stress and reprogramming of CNS gene transcription in development. Proceedings Of The National Academy Of Sciences, 110(13), 5169-5174. doi: 10.1073/pnas.1300065110

INSERM (Salle de presse. (2017, June 13). Prenatal stress affects life expectancy in offspring. Retrieved April 30, 2021, from Newsroom | Inserm website: https://presse.inserm.fr/en/le-stress-prenatal-affecte-la-longevite/28674/

Irizarry RA, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P et al (2009). The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. Nat Genet 41: 178–186.

Jaksic, N., Džananović, E. Š., Margetic, B. A., Rudan, D., Franc, A. C., Bozina, N., Bojić, E. F., Kučukalić, S., Kulenović, A. D., Marjanović, D., Avdibegović, E., Babić, D., Agani, F., Kučukalić, A., Mehmedbašić, A. B., Kravic, N., Umihanić, M. M., Sinanović, O., Babić, R., … Jakovljevic, M. (2019). A Candidate Gene Association Study of FKBP5 and CRHR1 Polymorphisms in Relation to War-Related Posttraumatic Stress Disorder - PubMed. Psychiatria Danubina, 31(2). https://doi.org/10.24869/psyd.2019.269

Jensen Peña, C., Monk, C., & Champagne, F. (2012). Epigenetic Effects of Prenatal Stress on 11β-Hydroxysteroid Dehydrogenase-2 in the Placenta and Fetal Brain. Plos ONE, 7(6), e39791. doi: 10.1371/journal.pone.0039791

Jr, H. P. S., Adynski, H., Harris, R., Bhattacharya, A., Rodriguez, A. C. I., Cali, R., Yabar, A. T., Nephew, B. C., & Murgatroyd, C. (2021). Biopsychosocial correlates of psychological distress in Latina mothers - PubMed. Journal of Affective Disorders, 282. https://doi.org/10.1016/j.jad.2020.12.193

Kallak, T., Bränn, E., Fransson, E., Johansson, Å., Lager, S., & Comasco, E. et al. (2021). DNA methylation in cord blood in association with prenatal depressive symptoms. Clinical Epigenetics, 13(1). doi: 10.1186/s13148-021-01054-0

Ke, X., Huang, Y., Fu, Q., Lane, R., & Majnik, A. (2021). Adverse Maternal Environment Alters MicroRNA-10b-5p Expression and Its Epigenetic Profile Concurrently with Impaired Hippocampal Neurogenesis in Male Mouse Hippocampus. Developmental Neuroscience, 1-11. doi: 10.1159/000515750

Kertes, D., Kamin, H., Hughes, D., Rodney, N., Bhatt, S., & Mulligan, C. (2016). Prenatal Maternal Stress Predicts Methylation of Genes Regulating the Hypothalamic–Pituitary–Adrenocortical System in Mothers and Newborns in the Democratic Republic of Congo. Child Development, 87(1), 61-72. doi: 10.1111/cdev.12487

Kertes, D., Bhatt, S., Kamin, H., Hughes, D., Rodney, N., & Mulligan, C. (2017). BNDF methylation in mothers and newborns is associated with maternal exposure to war trauma. Clinical Epigenetics, 9(1). doi: 10.1186/s13148-017-0367-x

Kimura, H. (2013). Histone modifications for human epigenome analysis. Journal of Human Genetics, 58(7), 439–445. https://doi.org/10.1038/jhg.2013.66

Kitraki, E., Karandrea, D., & Kittas, C. (1999). Long-Lasting Effects of Stress on Glucocorticoid Receptor Gene Expression in the Rat Brain. Neuroendocrinology, 69(5), 331–338. doi:10.1159/000054435

Khandelwal, N., Dey, S., Chakravarty, S., & Kumar, A. (2019). miR-30 Family miRNAs Mediate the Effect of Chronic Social Defeat Stress on Hippocampal Neurogenesis in Mouse Depression Model. Frontiers In Molecular Neuroscience, 12. doi: 10.3389/fnmol.2019.00188

Koolhaas, J., Bartolomucci, A., Buwalda, B., de Boer, S., Flügge, G., & Korte, S. et al. (2011). Stress revisited: A critical evaluation of the stress concept. Neuroscience & Biobehavioral Reviews, 35(5), 1291-1301. doi: 10.1016/j.neubiorev.2011.02.003

Laird, P. (2010). Principles and challenges of genome-wide DNA methylation analysis. Nature Reviews Genetics, 11(3), 191-203. doi: 10.1038/nrg2732

Lei, L., Wu, X., Gu, H., Ji, M., & Yang, J. (2020). Differences in DNA Methylation Reprogramming Underlie the Sexual Dimorphism of Behavioral Disorder Caused by Prenatal Stress in Rats. Frontiers in Neuroscience, 14, 573107. https://doi.org/10.3389/fnins.2020.573107

Leontiou, C., Hadjidaniel, M., Mina, P., Antoniou, P., Ioannides, M., & Patsalis, P. (2015). Bisulfite Conversion of DNA: Performance Comparison of Different Kits and Methylation Quantitation of Epigenetic Biomarkers that Have the Potential to Be Used in Non-Invasive Prenatal Testing. PLOS ONE, 10(8), e0135058. doi: 10.1371/journal.pone.0135058

Letourneau, N., Ntanda, H., Jong, V., Mahinpey, N., Giesbrecht, G., & Ross, K. (2021). Prenatal maternal distress and immune cell epigenetic profiles at 3-months of age. Developmental Psychobiology. doi: 10.1002/dev.22103

Lima, S., El Dib, R., Rodrigues, M., Ferraz, G., Molina, A., & Neto, C. et al. (2018). Is the risk of low birth weight or preterm labor greater when maternal stress is experienced during pregnancy? A systematic review and meta-analysis of cohort studies. PLOS ONE, 13(7), e0200594. doi: 10.1371/journal.pone.0200594

Lindsay, R. S., Lindsay, R. M., Waddell, B. J., & Seckl, J. R. (1996). Prenatal glucocorticoid exposure leads to offspring hyperglycaemia in the rat: studies with the 11 beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone - PubMed. Diabetologia, 39(11). https://doi.org/10.1007/s001250050573

Littlejohn, B. P., Price, D. M., Neuendorff, D. A., Carroll, J. A., Vann, R. C., Riggs, P. K., … Randel, R. D. (2018). Prenatal transportation stress alters genome-wide DNA methylation in suckling Brahman bull calves. Journal of Animal Science, 96(12), 5075–5099. https://doi.org/10.1093/jas/sky350

Littlejohn, B. P., Price, D. M., Neuendorff, D. A., Carroll, J. A., Vann, R. C., Riggs, P. K., … Welsh, T. H. (2020). Influence of prenatal transportation stress-induced differential DNA methylation on the physiological control of behavior and stress response in suckling Brahman bull calves. Journal of Animal Science, 98(1), skz368. https://doi.org/10.1093/jas/skz368

Liu, C. H., Erdei, C., & ; Mittal, L. (2021). Risk factors for depression, anxiety, and PTSD symptoms in perinatal women during the COVID-19 Pandemic. Psychiatry Research, 295, 113552. https://doi.org/10.1016/j.psychres.2020.113552

Li, Y. (2021). Modern epigenetics methods in biological research. Methods, 187, 104–113. https://doi.org/10.1016/j.ymeth.2020.06.022

Leuner, B., & Sabihi, S. (2016). The birth of new neurons in the maternal brain: Hormonal regulation and functional implications. Frontiers in Neuroendocrinology, 41, 99–113. https://doi.org/10.1016/j.yfrne.2016.02.004

Luft, C., Levices, I., Costa, M., Haute, G., Grassi-Oliveira, R., Oliveira, J., & Donadio, M. (2020). Exercise before pregnancy attenuates the effects of prenatal stress in adult mice in a sex-dependent manner. International Journal Of Developmental Neuroscience, 80(2), 86-95. doi: 10.1002/jdn.10001

Lu, Y., Jiang, J., Si, J., Wu, Q., Tian, F., Jiao, K., Mu, Y., Dong, P., & Zhu, Z. (2020). PDLIM5 improves depression-like behavior of prenatal stress offspring rats via methylation in male, but not female - PubMed. Psychoneuroendocrinology, 115. https://doi.org/10.1016/j.psyneuen.2020.104629

McCoy, C., Rana, S., Stringfellow, S., Day, J., Wyss, J., Clinton, S., & Kerman, I. (2016). Neonatal maternal separation stress elicits lasting DNA methylation changes in the hippocampus of stress reactive Wistar Kyoto rats. European Journal Of Neuroscience, 44(10), 2829-2845. doi: 10.1111/ejn.13404

Maccari, S. (2010). Perinatal Influences on Behavior and Neuroendocrine Functions. Encyclopedia of Behavioral Neuroscience, 35–39. https://doi.org/10.1016/b978-0-08-045396-5.00194-9

Martin, A., Pouchnik, D., Walker, J., & Wyrick, J. (2004). Redundant Roles for Histone H3 N-Terminal Lysine Residues in Subtelomeric Gene Repression in Saccharomyces cerevisiae. Genetics, 167(3), 1123-1132. doi: 10.1534/genetics.104.026674

Mansell, T., Novakovic, B., Meyer, B., Rzehak, P., Vuillermin, P., & Ponsonby, A. et al. (2016). The effects of maternal anxiety during pregnancy on IGF2/H19 methylation in cord blood. Translational Psychiatry, 6(3), e765-e765. doi: 10.1038/tp.2016.32

Matrisciano, F., Tueting, P., Dalal, I., Kadriu, B., Grayson, D. R., Davis, J. M., … Guidotti, A. (2013). Epigenetic modifications of GABAergic interneurons are associated with the schizophrenia-like phenotype induced by prenatal stress in mice. Neuropharmacology, 68, 184–194. https://doi.org/10.1016/j.neuropharm.2012.04.013

Mazzocchi, F. (2008). Complexity in biology. Exceeding the limits of reductionism and determinism using complexity theory. EMBO Reports, 9(1), 10–14. https://doi.org/10.1038/sj.embor.7401147

Meyer, J. S. (1985). Biochemical effects of corticosteroids on neural tissues - PubMed. Physiological Reviews, 65(4). https://doi.org/10.1152/physrev.1985.65.4.946

Meissner, A., Mikkelsen, T., Gu, H., Wernig, M., Hanna, J., & Sivachenko, A. et al. (2008). Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature, 454(7205), 766-770. doi: 10.1038/nature07107

Moore, L., Le, T., & Fan, G. (2012). DNA Methylation and Its Basic Function. Neuropsychopharmacology, 38(1), 23-38. doi: 10.1038/npp.2012.112

Monk, C., Feng, T., Lee, S., Krupska, I., Champagne, F., & Tycko, B. (2016). Distress During Pregnancy: Epigenetic Regulation of Placenta Glucocorticoid-Related Genes and Fetal Neurobehavior. American Journal Of Psychiatry, 173(7), 705-713. doi: 10.1176/appi.ajp.2015.15091171

Monteleone, M., Adrover, E., Pallarés, M., Antonelli, M., Frasch, A., & Brocco, M. (2013). Prenatal stress changes the glycoprotein GPM6A gene expression and induces epigenetic changes in rat offspring brain. Epigenetics, 9(1), 152-160. doi: 10.4161/epi.25925

Montoya-Williams, D., Quinlan, J., Clukay, C., Rodney, N. C., Kertes, D. A., & Mulligan, C. J. (2018). Associations between maternal prenatal stress, methylation changes in IGF1 and IGF2, and birth weight - PubMed. Journal of Developmental Origins of Health and Disease, 9(2). https://doi.org/10.1017/S2040174417000800

Morgan, C., & Bale, T. (2011). Early Prenatal Stress Epigenetically Programs Dysmasculinization in Second-Generation Offspring via the Paternal Lineage. Journal Of Neuroscience, 31(33), 11748-11755. doi: 10.1523/jneurosci.1887-11.2011

Mueller, B., & Bale, T. (2008). Sex-Specific Programming of Offspring Emotionality after Stress Early in Pregnancy. Journal Of Neuroscience, 28(36), 9055-9065. doi: 10.1523/jneurosci.1424-08.2008

Murgatroyd, C., Quinn, J., Sharp, H., Pickles, A., & Hill, J. (2015). Effects of prenatal and postnatal depression, and maternal stroking, at the glucocorticoid receptor gene. Translational Psychiatry, 5(5), e560-e560. doi: 10.1038/tp.2014.140

Mychasiuk, R., Ilnytskyy, S., Kovalchuk, O., Kolb, B., & Gibb, R. (2011). Intensity matters: brain, behaviour and the epigenome of prenatally stressed rats. Neuroscience, 180, 105-110. doi: 10.1016/j.neuroscience.2011.02.026

Nemoda, Z., & Szyf, M. (2017). Epigenetic Alterations and Prenatal Maternal Depression. Birth Defects Research, 109(12), 888-897. doi: 10.1002/bdr2.1081

Niknazar, S., Nahavandi, A., Peyvandi, A., Peyvandi, H., Zare Mehrjerdi, F., & Karimi, M. (2016). Effect of Maternal Stress Prior to Conception on Hippocampal BDNF Signaling in Rat Offspring. Molecular Neurobiology, 54(8), 6436-6445. doi: 10.1007/s12035-016-0143-5

Non, A., Binder, A., Kubzansky, L., & Michels, K. (2014). Genome-wide DNA methylation in neonates exposed to maternal depression, anxiety, or SSRI medication during pregnancy. Epigenetics, 9(7), 964-972. doi: 10.4161/epi.28853

Oberlander, T. F., Grunau, R., Mayes, L., Riggs, W., Rurak, D., Papsdorf, M., … Weinberg, J. (2008). Hypothalamic–pituitary–adrenal (HPA) axis function in 3-month old infants with prenatal selective serotonin reuptake inhibitor (SSRI) antidepressant exposure. Early Human Development, 84(10), 689–697. doi:10.1016/j.earlhumdev.2008.06.008

Oberlander, T., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., & Devlin, A. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. Epigenetics, 3(2), 97-106. doi: 10.4161/epi.3.2.6034

O'Donnell, Kieran & Janssen, Anna & Freeman, Laura & Khalife, Natasha & O'Connor, Thomas & Glover, Vivette. (2011). Maternal prenatal anxiety and downregulation of placental 11??-HSD2. Psychoneuroendocrinology. 37. 818-26. 10.1016/j.psyneuen.2011.09.014.

Ostlund, B. D., Conradt, E., Crowell, S. E., Tyrka, A. R., Marsit, C. J., & ; Lester, B. M. (2016). Prenatal Stress, Fearfulness, and the Epigenome: Exploratory Analysis of Sex Differences in DNA Methylation of the Glucocorticoid Receptor Gene. Frontiers in Behavioral Neuroscience, 10, 147. https://doi.org/10.3389/fnbeh.2016.00147

Palacios-García, I., Lara-Vásquez, A., Montiel, J., Díaz-Véliz, G., Sepúlveda, H., & Utreras, E. et al. (2015). Prenatal Stress Down-Regulates Reelin Expression by Methylation of Its Promoter and Induces Adult Behavioral Impairments in Rats. PLOS ONE, 10(2), e0117680. doi: 10.1371/journal.pone.0117680

Perrimon, N., Pitsouli, C., & Shilo, B.-Z. (2012). Signaling mechanisms controlling cell fate and embryonic patterning. Cold Spring Harbor Perspectives in Biology, 4(8), a005975 doi.org/10.1101/cshperspect.a005975

Perroud, N., Rutembesa, E., Paoloni-Giacobino, A., Mutabaruka, J., Mutesa, L., & Stenz, L. et al. (2014). The Tutsi genocide and transgenerational transmission of maternal stress: epigenetics and biology of the HPA axis. The World Journal Of Biological Psychiatry, 15(4), 334-345. doi: 10.3109/15622975.2013.866693

Peters, T. J., Buckley, M. J., Statham, A. L., Pidsley, R., Samaras, K., Lord, R. V., … Molloy, P. L. (2015). De novo identification of differentially methylated regions in the human genome. Epigenetics & Chromatin, 8(1), 1–16. https://doi.org/10.1186/1756-8935-8-6

Pradhan, S., Bacolla, A., Wells, R., & Roberts, R. (1999). Recombinant Human DNA (Cytosine-5) Methyltransferase. Journal Of Biological Chemistry, 274(46), 33002-33010. doi: 10.1074/jbc.274.46.33002

Provençal, N., Arloth, J., Cattaneo, A., Anacker, C., Cattane, N., Wiechmann, T., … Binder, E. B. (2020). Glucocorticoid exposure during hippocampal neurogenesis primes future stress response by inducing changes in DNA methylation. Proceedings of the National Academy of Sciences, 117(38), 23280–23285. https://doi.org/10.1073/pnas.1820842116

Rijlaarsdam, J., van IJzendoorn, M., Verhulst, F., Jaddoe, V., Felix, J., Tiemeier, H., & Bakermans-Kranenburg, M. (2016). Prenatal stress exposure, oxytocin receptor gene (OXTR) methylation, and child autistic traits: The moderating role ofOXTRrs53576 genotype. Autism Research, 10(3), 430-438. doi: 10.1002/aur.1681

Schraut, K., Jakob, S., Weidner, M., Schmitt, A., Scholz, C., & Strekalova, T. et al. (2014). Prenatal stress-induced programming of genome-wide promoter DNA methylation in 5-HTT-deficient mice. Translational Psychiatry, 4(10), e473-e473. doi: 10.1038/tp.2014.107

Serpeloni, F., Radtke, K. M., Hecker, T., Sill, J., Vukojevic, V., Assis, S. G. de, … Nätt, D. (2019). Does Prenatal Stress Shape Postnatal Resilience? – An Epigenome-Wide Study on Violence and Mental Health in Humans. Frontiers in Genetics, 10. https://doi.org/10.3389/fgene.2019.00269

Sharifi-Zarchi, A., Gerovska, D., Adachi, K., Totonchi, M., Pezeshk, H., & Taft, R. et al. (2017). DNA methylation regulates discrimination of enhancers from promoters through a H3K4me1-H3K4me3 seesaw mechanism. BMC Genomics, 18(1). doi: 10.1186/s12864-017-4353-7

Shen, L., Kondo, Y., Guo, Y., Zhang, J., Zhang, L., & Ahmed, S. et al. (2007). Genome-Wide Profiling of DNA Methylation Reveals a Class of Normally Methylated CpG Island Promoters. Plos Genetics, 3(10), e181. doi: 10.1371/journal.pgen.0030181

Sjaarda, C., Hecht, P., McNaughton, A., Zhou, A., Hudson, M., & Will, M. et al. (2017). Interplay between maternal Slc6a4 mutation and prenatal stress: a possible mechanism for autistic behavior development. Scientific Reports, 7(1). doi: 10.1038/s41598-017-07405-3

Song, J. et al. Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. Nature489, 150–154, https://doi.org/10.1038/nature11306 (2012).

Solomonova, E., Lee, Y., Robins, S., King, L., Feeley, N., & Gold, I. et al. (2019). Sleep quality is associated with vasopressin methylation in pregnant and postpartum women with a history of psychosocial stress. Psychoneuroendocrinology, 107, 160-168. doi: 10.1016/j.psyneuen.2019.05.010

Sosnowski, D. W., Booth, C., York, T. P., Amstadter, A. B., & Kliewer, W. (2018). Maternal prenatal stress and infant DNA methylation: A systematic review - PubMed. Developmental Psychobiology, 60(2). https://doi.org/10.1002/dev.21604

Stevens, H. E., Su, T., Yanagawa, Y., & Vaccarino, F. M. (2013). Prenatal stress delays inhibitory neuron progenitor migration in the developing neocortex. Psychoneuroendocrinology, 38(4), 509–521. https://doi.org/10.1016/j.psyneuen.2012.07.011

Stroud, L., Papandonatos, G., Parade, S., Salisbury, A., Phipps, M., & Lester, B. et al. (2016). Prenatal Major Depressive Disorder, Placenta Glucocorticoid and Serotonergic Signaling, and Infant Cortisol Response. Psychosomatic Medicine, 78(9), 979-990. doi: 10.1097/psy.0000000000000410

Szyf, M. (2019). The epigenetics of perinatal stress. Dialogues in Clinical Neuroscience, 21(4), 369–378. https://doi.org/10.31887/DCNS.2019.21.4/mszyf

Tang, L. (2019). Bisulfite-free epigenetic sequencing. Nature Methods, 16(4), 286-286. doi: 10.1038/s41592-019-0387-x

Trump, S., Bieg, M., Gu, Z., Thürmann, L., Bauer, T., & Bauer, M. et al. (2016). Prenatal maternal stress and wheeze in children: novel insights into epigenetic regulation. Scientific Reports, 6(1). doi: 10.1038/srep28616

Unnikrishnan, A., Gafken, P., & Tsukiyama, T. (2010). Dynamic changes in histone acetylation regulate origins of DNA replication. Nature Structural & Molecular Biology, 17(4), 430-437. doi: 10.1038/nsmb.1780

Unternaehrer, E., Bolten, M., Nast, I., Staehli, S., Meyer, A., & Dempster, E. et al. (2016). Maternal adversities during pregnancy and cord blood oxytocin receptor (OXTR) DNA methylation. Social Cognitive And Affective Neuroscience, 11(9), 1460-1470. doi: 10.1093/scan/nsw051

Valensisi, C., Liao, J. L., Andrus, C., Battle, S. L., & Hawkins, R. D. (2015). cChIP-seq: a robust small-scale method for investigation of histone modifications. BMC Genomics, 16(1), 1–11. doi.org/10.1186/s12864-015-2285-7

Vangeel, E. B., Izzi, B., Hompes, T., Vansteelandt, K., Lambrechts, D., Freson, K., & Claes, S. (2015). DNA methylation in imprinted genes IGF2 and GNASXL is associated with prenatal maternal stress - PubMed. Genes, Brain, and Behavior, 14(8). https://doi.org/10.1111/gbb.12249

Vangeel, E., Pishva, E., Hompes, T., van den Hove, D., Lambrechts, D., & Allegaert, K. et al. (2017). Newborn genome-wide DNA methylation in association with pregnancy anxiety reveals a potential role for GABBR1. Clinical Epigenetics, 9(1). doi: 10.1186/s13148-017-0408-5

Van den Hove, D., Kenis, G., Brass, A., Opstelten, R., Rutten, B., & Bruschettini, M. et al. (2013). Vulnerability versus resilience to prenatal stress in male and female rats; Implications from gene expression profiles in the hippocampus and frontal cortex. European Neuropsychopharmacology, 23(10), 1226-1246. doi: 10.1016/j.euroneuro.2012.09.011

Van Regenmortel, M. H. V. (2004). Reductionism and complexity in molecular biology. Scientists now have the tools to unravel biological and overcome the limitations of reductionism. EMBO Reports, 5(11), 1016–1020. https://doi.org/10.1038/sj.embor.7400284

Vidal, A. C., Neelon, S. E. B., Liu, Y., Tuli, A. M., Fuemmeler, B. F., Hoyo, C., … Murphy, S. K. (2014). Maternal Stress, Preterm Birth, and DNA Methylation at Imprint Regulatory Sequences in Humans. Genetics & Epigenetics, 6, GEG.S18067. https://doi.org/10.4137/geg.s18067

Von Bohlen Und Halbach, O. Immunohistological markers for staging neurogenesis in adult hippocampus. Cell Tissue Res329, 409–420, https://doi.org/10.1007/s00441-007-0432-4 (2007)

Welberg, L. A., & Seckl, J. R. (2001). Prenatal stress, glucocorticoids and the programming of the brain. Journal of neuroendocrinology, 13(2), 113–128. https://doi.org/10.1046/j.1365-2826.2001.00601.x

Wikenius, E., Myhre, A. M., Page, C. M., Moe, V., Smith, L., Heiervang, E. R., … LeBlanc, M. (2019). Prenatal maternal depressive symptoms and infant DNA methylation: a longitudinal epigenome-wide study. Nordic Journal of Psychiatry, 1–7. doi:10.1080/08039488.2019.1613446

Wu, S., Gennings, C., Wright, R., Wilson, A., Burris, H., & Just, A. et al. (2018). Prenatal Stress, Methylation in Inflammation-Related Genes, and Adiposity Measures in Early Childhood: the Programming Research in Obesity, Growth Environment and Social Stress Cohort Study. Psychosomatic Medicine, 80(1), 34-41. doi: 10.1097/psy.0000000000000517

Xu, L., Sun, Y., Gao, L., Cai, Y., & Shi, S. (2014). Prenatal Restraint Stress is Associated with Demethylation of Corticotrophin Releasing Hormone (CRH) Promoter and Enhances CRH Transcriptional Responses to Stress in Adolescent Rats. Neurochemical Research, 39(7), 1193-1198. doi: 10.1007/s11064-014-1296-0

Ye, D., Zhang, L., Fan, W., Zhang, X., & Dong, E. (2018). Genipin normalizes depression-like behavior induced by prenatal stress through inhibiting DNMT1. Epigenetics, 13(3), 310-317. doi: 10.1080/15592294.2018.1450033

Yong, W., Hsu, F., & Chen, P. (2016). Profiling genome-wide DNA methylation. Epigenetics & Chromatin, 9(1). doi: 10.1186/s13072-016-0075-3

Yuan, J., Yang, F., Chen, B., Lu, Z., Huo, X., & Zhou, W. et al. (2011). The histone deacetylase 4/SP1/microrna-200a regulatory network contributes to aberrant histone acetylation in hepatocellular carcinoma. Hepatology, 54(6), 2025-2035. doi: 10.1002/hep.24606

Zentner, G., & Henikoff, S. (2013). Regulation of nucleosome dynamics by histone modifications. Nature Structural & Molecular Biology, 20(3), 259-266. doi: 10.1038/nsmb.2470

Zhang, X., Zhang, H., Liang, X., Liu, Q., Wang, H., & Cao, B. et al. (2016). Epigenetic mechanism of maternal post-traumatic stress disorder in delayed rat offspring development: dysregulation of methylation and gene expression. Genetics And Molecular Research, 15(3). doi: 10.4238/gmr.15039009

Zhao, F., & Bapat, B. (2016). The Role of Methylation-Specific PCR and Associated Techniques in Clinical Diagnostics. Epigenetic Biomarkers and Diagnostics, 155–173. https://doi.org/10.1016/b978-0-12-801899-6.00008-5

Zheng, Y., Fan, W., Zhang, X., & Dong, E. (2016). Gestational stress induces depressive-like and anxiety-like phenotypes through epigenetic regulation of BDNF expression in offspring hippocampus. Epigenetics, 11(2), 150-162. doi: 10.1080/15592294.2016.1146850

Zucchi, F., Yao, Y., Ward, I., Ilnytskyy, Y., Olson, D., & Benzies, K. et al. (2013). Maternal Stress Induces Epigenetic Signatures of Psychiatric and Neurological Diseases in the Offspring. Plos ONE, 8(2), e56967. doi: 10.1371/journal.pone.0056967

Zhu, C., Liang, M., Li, Y., Feng, X., Hong, J., & Zhou, R. (2018). Involvement of Epigenetic Modifications of GABAergic Interneurons in Basolateral Amygdala in Anxiety-like Phenotype of Prenatally Stressed Mice. International Journal Of Neuropsychopharmacology, 21(6), 570-581. doi: 10.1093/ijnp/pyy006

Supplementary data Table 1 Animal studies

Author	Category	Subcategory	Methods	Candidate gene (s)	Associated Phenotype
(Morgan & - Bale, 2011)	Mouse C57BL/6	Comparative study & miRNAs	Custom Taqman qRT-PCR Array, Tail suspension test.	reduced expression of miR-322, miR-574-3p,and miR-873, glycan expression was significantly increased inthe F2-S male PN1 brain.	observed a broad shift inexpression from a male-typical to a more female-typical patternin the F2 male offspring of prenatally stressed sires (F2-S). These develop-mental effects were associated with the transmission of a stress-sensitive phenotype and shortened anogenital distance in adult F2-Smales
(Littlejohn et al., 2019)	Mature Brahman cows	Comparative Study, DNA methylation	bisulfite sequencing methods, Methyl-MiniSeq	Among differentially methylated genes ($P \leq 0.05$) related to behavior and the stress response were OPRK1, OPRM1, PENK, POMC, NR3C2, TH, DRD1, DRD5, COMT, HTR6, HTR5A, GABRA4, GABRQ, and GAD2	elevations in temperament score and serum cortisol through weaning in the larger population of PNS calves from which bulls in this study were derived.
(Palacios-García et al., 2015)	Rats	Comparison study, DNA methylation	western blot and DNA methylation assay - Conventional PCR and behavioral tests	Methylation of the Reelin gene promoter is increased in the cortex of PNS new born rats	PNS adult rats display excessive spontaneous locomotor activity, high anxiety levels and problems of learning and memory consolidation. No significant visuo-spatial memory impairment was detected on the Morris water maze
(Jensen Peña, Monk, & Champagne, 2012)	Long Evans Rats	Behavioral study, DNA methylation	Purified DNA was analyzed (EpigenDX) for CpG methylation by bisulfite pyrosequencing	1β-hydroxysteroid dehydrogenase type 2 (HSD11B2) gene promoter,DNA methyltransferase DNMT3a.	Within individuals, they identified CpG sites within the HSD11B2 gene promoter and exon 1 at which DNA methylation levels were highly correlated between the placenta and fetal cortex.

(Luft et al., 2020)	Mouse	Comparatice study, Histone modifications	EpiQuiK Global Histone H3 Acetylation Assay Kit	glucocorticoid receptor (GR) gene expression decrease in the prefrontal cortex in PNS males, as well as a histone H3 acetylation decrease (p = .06) close to the significance level.	When the HDAC activity was assessed, no significant differences were found between all experimental .nearly significant (p = .06) basal corticosterone increase was observed in PNS males and the exercise before pregnancy reduced the stress-induced corticosterone increase in PNS females. In addition, an increase on prefrontal cortex (PFC) CRHR1 gene expression was observed in PNS females, which was attenuated by the exercise before gestation
(Ye, Zhang, Fan, Zhang, & Dong, 2018)	Mouse model , kumming	Preclinical study, DNA methylation	methylated DNA immunoprecipitation and qPCR	BDNF- Brain derived Neurotrophic factor and DNMT1	Stress exposure during pregnancy increased DNMT1 expression and decreased the expression of hippocampal BDNF due to promoter methylation.
(Sjaarda et al., 2017)	Mouse	Scientific report, Genome wide DNA methylation	Whole genome profiling of methylome, transcriptome and miRNA using Next Generation Sequencing	Embryos of stressed Slc6a4 (+/+) dams exhibited significantly altered methylation profiles and differential expression of 157 miRNAs and 1009 genes affecting neuron development and cellular adhesion pathways, which may function as a coping mechanism to prenatal stress.	The response of embryos of stressed Slc6a4 (+/-) dams was found to be attenuated, shown by significantly reduced numbers of differentially expressed genes (458) and miRNA (0) and genome hypermethylatio
(Monteleone et al., 2013)	Wistar rats	Locus specific DNA methylation and miRNA	retrotranscription and qPCR to identify miRNA levels and , bisulfite conversion,	gpm6a gene in the hippocampus whose function is involved in neurite outgrowth, filipodium/spine formation, filipidium motility and synapse formation.microRNA-133b showed that miR-133 overexpression affected gpm6a mRNA and GPM6A protein levels	Prenatal stress induced changes in gpm6a levels in both tissues and at both ages analyzed, indicating a persistent effect. microRNA-133b was the most significantly altered.
(Benoit, Rakic, & Frick, 2015)	Mouse	Association study, both locus DNA methylation & Histone modification	Measure levels of DNMT1 protein and levels of acetylated H3 at lysine-14 (AcH3Lys14) using western blotting	Acetylated histone H3 (Lys 14) levels were reduced in the dorsal hippocampus by PNS in both sexes, but significantly more so for the female PNS group and levels of the maintenance methyltransferase DNMT1 are increased by PNS in females only.	prenatal chronic unpredictable stress generally impaired spatial memory in the Morris water maze is in agreement with previous work using chronic immobilization stress

(Dong et al., 2015)	Mouse	Preclinical study, AHistone and DNA methylation	Methylated DNA immunoprecipitation Methylated and hydroxymethylated DNA on Bdnf exon I to IX were assessed using MeDIP and hMeDIP kits and d chromatin immunoprecipitation (ChIP) assays .	DNA methyltransferase 1 and ten-eleven-translocation hydroxylase 1 in the frontal cortex and hippocampus	Adult PRS offspring demonstrate behavioral abnormalities suggestive of SZ and molecular changes similar to changes seen in postmortem brains of patients with SZ. This includes a significant increase in DNA methyltransferase 1 and ten-eleven-translocation hydroxylase 1 in the frontal cortex and hippocampus but not in cerebellum; no changes in histone deacetylases, histone methyltransferases and demethylases, or methyl CpG binding protein 2, and a significant decrease in Bdnf messenger RNA variants.
(Mychasiuk, Ilnytskyy, Kovalchuk, Kolb, & Gibb, 2011)	Rats	Genome wide association study, DNA methylation	qRT-PCR	mild prenatal stress increased global DNA methylation levels in the frontal cortex and hippocampus whereas high prenatal stress was associated with a dramatic decrease.	The two different prenatal stress intensities produced significantly different and often, opposite effects in the developing brain. Mild prenatal stress decreased brain weight in both males and females, whereas extreme stress increased female brain weight. Mild prenatal stress slowed development of sensorimotor abilities and decreased locomotion, whereas high prenatal stress also slowed development of sensorimotor learning but increased locomotion.
(Cattaneo et al., 2020)	Rats and Human study	Comparative study , miRNA-30a	Methylated DNA immunoprecipitation (MeDIP) analysis and Gene and miRNA expression analyses by Real Time PCR	miR-30a and Neurod1 emerged as potential players for the negative outcomes associated with PNS exposure. also found a significant reduction in the expression of a panel of genes (CAMK2A, c-JUN, LIMK1, MAP2K1, MAP2K2, PIK3CA and PLCG1) that belong to these two biological pathways and are also validated targets of miR-30a, pointing to a down-regulation of these pathways as a consequence of PNS exposure.	found that the methylation status of the miR-30a gene is significantly reduced in the HIP and PFC of adult male and female rats whose mothers had been exposed to stress during the last week of gestation, as compared to controls and also found that miR-30a-5p levels were significantly elevated in the blood of depressed patients who experienced traumatic events early in life, as compared to controls

(Cilkiz et al., 2020)	Brahman heifer calves	Longitudinal study , DNA methylation	reduced representation bisulphite sequencing	173 differentially methylated CpG sites within gene body regions in PNS relative to Control cows, Solute carrier family 5 member 2 (SLC5A2), Junctophilin 2 (JPH2), Sialic acid binding Ig like lectin 15 (SIGLEC15), Goosecoid homeobox (GSC), Insulin-like growth factor binding protein 1 (IGFBP1), LRAT domain containing 1 (LRATD1), CXADR like membrane protein (CLMP)	Genome-wide DNA methylation differences in lymphocytes of female Brahman cattle due to age and prenatal stress were substantial.
(Zucchi et al., 2013)	Rats	Comparative study, non coding RNA (miRNA)	Quantutative real time PCR and gene microarray expression analysis	prenatal stress upregulated miR-103 which downregulated its potential gene target Ptplb. PNS upregulated miR-145, miR-323 ,miR-98, miR-219, which targets the gene Dazap1.	Offspring transcriptomic changes included genes related to development, axonal guidance and neuropathology
(Boersma et al., 2014)	Rats	Association locus specific DNA methylation study	Bisulfite pyrosequencing Genomic DNA	BDNF increased methylation. (Dnmt) 1 and 3a expression was increased in PNS rats in the amygdala and hippocampus.	Observed decreased Bdnf expression in the amygdala and hippocampus of prenatally stressed rats both at weaning and in adulthood
(Ke, Huang, Fu, Lane, & Majnik, 2021)	Mouse C57/Bl6	Association study , non coding RNA (miRNA)	RT-PCR, pyrosequencing and CHIP Chromatin Immunoprecipitation Assay	AME significantly increased hippocampal miR-10b-5p level, decreased DNA methylation and increased accumulations of active histone marks H3 lysine (K) 4me3, H3K14ac, and -H3K36me3 at miR-10b promoter.	Decreased hippocampal neurogenesis by low numbers of proliferation markers, neuronal differentiation markers and decreased hippocampal BDNF levels
(Dong, Tueting, Matrisciano, Grayson, & Guidotti, 2016)	Mouse	Comparison study, DNA methylation	Methylated DNA immunoprecipitation using MeDIP , ChIP assays	Increases in 5MC and 5HMC at promoter regions corresponding to Gad1 glutamic acid decarboxylase 67 , Reln (Reelin) and Bdnf (brain derived neurotrophic factors) and reduction in mRNA and protein expression. These genes are correlated with locomotor activity and social interaction.	Positive symptoms (stereotype behaviors, sensitivity to NMDA receptor antagonists and Cognitive, information processing deficit (PPI, fear conditioning)

(Schraut et al., 2014)	Mouse , C57BL6/J	Locus specific methylation comparison study, DNA methylation	Genome-wide hippocampal DNA methylation screening using methylated-DNA immunoprecipitation (MeDIP) on Affymetrix GeneChip Mouse Promoter 1.0 R arrays	Differentially methylated region in the gene encoding myelin basic protein (Mbp) was associated with its expression in a 5-Htt-, PS- and 5-Htt × PS-dependent manner	increased depression-like behavior in the forced-swim test, an effect that was particularly pronounced in female offspring.
(Blaze et al., 2017)	Rats	Association study, locus DNA methylation analysis	DNA was bisulfite-converted (Epitect Bisulfite Kit, Qiagen, Inc., Valencia, CA) and direct bisulfite-sequencing PCR (BSP) was	Bdnf IV DNA methylation	males from prenatally stressed mothers had significantly higher levels of methylation in the mPFC compared to male controls and females from prenatally stressed mothers. also found that prenatally stressed animals exhibited shorter telomeres in the mPFC compared to controls.
(Lu et al., 2020)	Rats	Locus specific methylation analysis &DNA methytion	qRT-PCR and western blot, respectively. The methylation of PDLIM5 promoter were analyzed by bisulfite sequencing	Methylation level of PDLIM5 promoter showed a significant gender difference in PS offspring rats. PDLIM5 mainly locates in the presynaptic nerve endings of hippocampal neurons and is closely related to the release of neurotransmitters	PS offspring rats showed a significant decrease in sucrose preference and a prolonged immobility time. Injection of PDLIM5 significantly improved the depression-like behavior in PS offspring rats, whereas administration of PDLIM5 shRNA aggravated it
(Howerton, Morgan, Fischer, & Bale, 2013)	Mouse	Genome wide association study, miRNA modification	Expression levels of 245 microRNAs were determined using the Taqman Array MicroRNA card A Array (Applied Biosystems).	O-linked-N-acetylglucosamine (O-GlcNAc) transferase (OGT) O-GlcNAcylation, were significantly lower in males and further reduced by prenatal stress. robust differences in the brain microRNA environment, where hemizygous placental OGT expression shifted the pattern of 250 of the most abundant brain microRNAs to be distinct from that of wild-type females by hierarchical clustering analyses	studies identified OGT, an intracellular glycotransferase important in regulating key chromatin programming events, as a potential placental biomarker that was sex-biased in its expression, responsive to EPS, and similar in X linked expression pattern in human tissue.

(Zheng, Fan, Zhang, & Dong, 2016)	Mouse	DNA methylation, and histone modifications	RT-qPCR, Western blot , ChIP, methylated DNA immunoprecipitation (MeDIP)	stress-offspring showed decreased expression of BDNF, increased expression of DNMT1, HDAC1, and HDAC2, and decreased expression of AcH3K14 in the hippocampus as compared to non-stress offspring.	results show that offspring from gestational stress dams exhibited depressive-like and anxiety-like behaviors
(Lei, Wu, Gu, Ji, & Yang, 2020)	Rats	Preclinical study, Locus specific methylation analysis	pyrosequencing, western blotting, and Golgi staining to assess changes in methylation	:he methylation pattern in the promoter region of the GR gene differed between males and females. Sex-specific changes in the expression of DNMTs (DNMT1 and DNMT3a) and DNA demethylase (Tet methylcytosine dioxygenase 2) were also observed	results showed that PS increased anxiety-like behavior in offspring, especially in females, while depression-like behavior was increased in male offspring compared to control littermates
(Zhu et al., 2018)	Mice	Preclinical study , DNA methylation and Histone modificayions	. Real-time RT-PCR, western blot, chromatin immunoprecipitation, and electrophysiological analysis were employed to detect	The decrease of glutamic acid decarboxylase 67 transcript was paralleled by an enrichment of 5-methylcytosine in glutamic acid decarboxylase 67 promoter regions.	mice developed an anxiety-like phenotype accompanied by a significant increase of DNA methyltransferase 1 and a reduced expression of glutamic acid decarboxylase 67 in the basolateral amygdala.
(Van den Hove et al., 2013)	Rats	Preclinical study, Histone modification	Microarray-based profiling	PS affected the expression of 44 and 1084 genes in male and female offspring, respectively. Within the FC, 114 and 688 genes were expressed differentially by PS in male and female offspring	PS significantly increased anxiety-related behavior in male, but not female offspring. Likewise, depression-related behavior was increased in male PS rats only. Further, male PS offspring showed increased basal plasma corticosterone levels in adulthood, whereas both PS males and females had lower stress-induced corticosterone levels when compared to controls
(Zhang et al., 2016)	Rats	Genome wide association study, DNA methylation	Open-field tests (OFTs), elevated pull maze (EPM) assays, gene expression profile chip tests, and methylated DNA immunoprecipitation sequencing (MeDIP-Seq) were performed on	DNA methylation levels in 1675 CGs of 957 genes; this was primarily correlated with various immune functions, although maternal subjective distress was not correlated. DNA methylation in SCG5 and LTA is highly associated with maternal objective stress and is comparable in peripheral blood T cells, mononuclear cells, and saliva cells . Itgb6 was the most frequently identified gene in the identified pathways (4/9)	In a comparison of the PTSD group versus the control group, 4,160 significantly differentially methylated loci containing 30,657 CpGs were identified; 2,487 genes, including 13 dysmethylated genes, were validated by gene expression profiling, showing a negative correlation between methylation and gene expression (R = -0.617, P = 0.043).

(Niknazar et al., 2016)	Rats	Compartive study, DNA methylation locus specific	DNF and TrkB gene methylation and protein expression in the hippocampus were detected using real-time PCR and Western blotting in all groups	Brain-derived neurotrophic factor (BDNF) s the important regulator of neural survival, development, and its genetic and epigenetic alterations which have been linked with several neuropsychiatric disorders and TrkB promoter hypermethylation CpG2 site in frontal cortex is accompanied by suicidal behavior and leads to decreased expression of TrkB mRNA	hirty-day-old male and female pups from SM groups had a significantly more serum corticosterone concentration, DNA methylation levels of BDNF and TrKB, and lower expression of these genes compared to pups from the control groups. Male pups from stressed mother exhibited significant anxiety-like behavior compared to male pups from the control mothers
(Mueller & Bale, 2008)	Mouse	behavioral study, GWA DNA methyati	Pyrosequencing was performed by EpigenDx, TaqMan Gene Expression Assay	Changes in CRF and GR gene methylation correlated with altered gene expression, providing important evidence of epigenetic programming during early prenatal stress	found that male offspring exposed to stress early in gestation displayed maladaptive behavioral stress responsivity, anhedonia, and an increased sensitivity to selective serotonin reuptake inhibitor treatment. Long-term alterations in central corticotropin-releasing factor (CRF) and glucocorticoid receptor (GR) expression, as well as increased hypothalamic–pituitary–adrenal (HPA) axis responsivity, were
(Xu, Sun, Gao, Cai, & Shi, 2014)	Spragye-Dawley rats	Locus specific DNA methylation study	Genomic DNA Extraction and Bisulfite Sequencing	demethylation of CpG dinucleotides in the CRH promoter. observed that PRS is associated with reduced methylation of the CpG dinucleotides in the CRH promoter.	results showed that prenatal stress is associated with the demethylation of the CRH promoter, and leads to anxietylike behaviors in adolescent life stages, as well as hyperresponsiveness of the HPA axis

Supplementary Table 2 Human Studies

Author	Category	Subcategory	Methods	Candidate genes	Associated Phenotypes
(Oberlander et al., 2008)	Human	Assosiation study, locus specific DNA methylation	bisulfite [pyrosequencing.	increased methylation of NR3C1 at a predicted NGFI-A binding site	Increased NR3C1 methylation at this site was also associated with increased salivary cortisol stress responses at 3 months, controlling for prenatal SRI exposure, postnatal age and pre and postnatal maternal mood.
(Cao-Lei et al., 2015)	Human	longitudinal study, Global DNA methylation	Infinium Human Methylation 450 BeadChip Array	LTA, NFKBIA, and PIK3CD. LTA had the greatest number of significant CpGs, which are involved in both Type-1 and -2 diabetes mellitus pathways	methylation level of genes from established Type-1 and -2 diabetes mellitus pathways showed significant mediation of the effect of objective PNMS on both central adiposity and BMI
(Braithwaite, Kundakovic, Ramchandani, Murphy, & Champagne, 2015)	human	Birth Cohort, locus specific DNA methylation study	DNA methylation at specific CpG sites was analyzed using the quantitative bisulfite-pyrosequencing method using PyroMark Q24 pyrosequencer	*NR3C1 1F* and *BDNF IV* DNA methylation	Prenatal depressive symptoms significantly predicted increased *NR3C1 1F* DNA methylation in male infants and predicted decreased *BDNF IV* DNA methylation in both male and female infants. No measure of maternal cortisol during pregnancy predicted infant *NR3C1 1F* or *BDNF* promoter IV DNA methylation.
(Cao-Lei et al., 2014)	Human	Follow up comparative study, DNA methylation	Infinium Human Methylation 450 BeadChip Array,Bisulfite treatment and pyrosequencing	DNA methylation changes in *SCG5* , proprotein convertase *PC2* and lymphotoxin alpha *LTA* which are regulating the innate and adaptive immune system	Prenatal maternal objective hardship was correlated with DNA methylation levels in 1675 CGs affiliated with 957 genes predominantly related to immune function; maternal subjective distress was uncorrelated
(Non, Binder, Kubzansky, & Michels, 2014)	Human	GWAS , DNA methylation study	Illumina Infinium Human Methylation450 BeadChip	*NFKB2* , one site in *SLC6A4* , a marginally significant association with SSRI exposure was found at one site in *DNMT3a* , *FKBP5* , *NR3C* , and *CRHR11* .	42 CpG sites with significantly different DNA methylation levels in neonates exposed to non-medicated depression or anxiety relative to controls. In neonates exposed either to non-medicated maternal depression or SSRIs, the vast majority of CpG sites displayed lower DNA methylation relative to the controls, but differences were very small.

(Kertes et al., 2017)	Human	Cohort, Locus specific DNA methylation	Epigenotyping using Illumina HumanMethylation450 BeadChips and Sodium bisulfate sequencing	BDNF methylation at CpG sites	Associations of maternal stress and BDNF methylation showed high tissue specificity. The majority of significant associations were observed in putative transcription factor binding regions.
(Kertes et al., 2016)	Human	Association study, DNA methylation, locus specific	Illumina HumanMethylation450 BeadChips	CRH, CRHBP, NR3C1, and FKBP5 methylation with prenatal stress.	chronic stress and war trauma had widespread effects on HPA axis gene methylation, with significant effects observed at transcription factor binding (TFB) sites in all target genes tested
(Stroud et al., 2016)	Human	Descriptive study, Locus specific DNA methylation	bisulfite Pyrosequencing System (Qiagen)	*HSD11B2* methylation moderated links between prenatal MDD and baseline cortisol (p = .02), with 1% methylation decreases associated with 9% increased baseline cortisol in infants of prenatal MDD mothers (ratio = 1.09; 95% CI = 1.01-1.16). *SLC6A4* expression moderated links between prenatal MDD and cortisol response among boys alone (p = .007),	Placental *HSD11B2* methylation and *SLC6A4* gene expression moderated the influence of prenatal MDD on infant cortisol regulation. All findings were influenced by fetal sex. Effects of prenatal MDD on infant cortisol and modulating effects of *HSD11B2* were strongest for newborn daughters, while moderating effects of *SLC6A4* emerged only in sons.
Kim, Kim, Shin, & Kim, 2018)	Human	Prospective study, Locus specific DNA methylation	bisulfite sequencing procedure was performed to determine the CpG methylation profiles located upstream of the DRD4 coding	methylation of CpG7 the methylation of 28 cytosine-guanine dinucleotide (CpG) sites of *DRD4*.	There were no significant sites that showed significant association with treatment response,but there was a significant interaction effect of the methylation of CpG7 and prenatal maternal stress on changes in omission errors of the CPT following treatment (p = 0.0001).
(Duis et al., 2018)	Human	Birth cohort, Locus specific DNA methylation analysis	Bisulfite Pyrosequencing	SNP: rs1360780) in *FKBP5* (FK506 Binding Protein 5) has been shown to interact with exposure to childhood adversity to promote loss of methylation and increase in gene expression in adults	observed a significant recessive genotype effect with increased methylation at a subset of the five CpGs located in intron 5.

(Conradt, Lester, Appleton, Armstrong, & Marsit, 2013)	Human	Birth Cohort, Locus specific DNA methylation	Bisulfite pyrosequencing DNA methylation analysis	Exposure to maternal mood disorder in utero may program infant neurobehavior via DNA methylation of the glucocorticoid receptor (NR3C1) and 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2), two placental genes that have been implicated in perturbations of the hypothalamic pituitary adrenocortical (HPA) axis	infants whose mothers reported anxiety during pregnancy and showed greater methylation of placental 11β-HSD-2 CpG4 were more hypotonic compared with infants of mothers who did not report anxiety during pregnancy.
(Rijlaarsdam et al., 2017)	Human	Prospective study, Locus specific DNA methylation	Illumina Infinium HumanMethylation450 BeadChips	*OXTR* rs53576 genotype Prenatal maternal stress exposure was uniquely associated with child autistic traits but was unrelated to *OXTR* methylation across both *OXTR* rs53576 G-allele homozygous children and A-allele carriers.	For child autistic traits in general and social communication problems in particular, we observed a significant OXTR rs53576 genotype by OXTR methylation interaction in the absence of main effects, suggesting that opposing effects cancelled each other out.
(Letourneau et al., 2021)	Human	Birth Cohort, Locus DNA methylation study	IlluminaHumanMethylation450 array	methylation sites were generally in pathways that involved zinc finger proteins, which are involved in binding DNA and related molecules during transcription	Prenatal maternal distress did emerge as predictive of child immune DNA methylation patterns, independent of child sex, maternal sociodemographic variables, pre-pregnancy BMI, and postpartum distress symptoms.
(Unternaehrer et al., 2016)	Human	Cross sectional study, Locus specific DNA methylation	OXTR DNA methylation was quantified using Sequenom EpiTYPER	oxytocin receptor (OXTR)	The number of stressful life events (P = 0.032), EPDS score (P = 0.007) and cortisol AUCgs at T2 (CAR: P = 0.020; DAY: P = 0.024) were negatively associated with OXTR DNA methylation.

(Vangeel et al., 2015)	Human	Birth cohort, Locus specific DNA methylation study	Sequenom EpiTYPER.	insulin-like growth factor 2 *(IGF2)* and guanine nucleotide-binding protein, alpha stimulating extra-large *GNASXL* showed DMR at CpG5 , P < 0.0001. *GNASXL* (CpG11, P = 0.0003), while *IGF2AS* was associated with maternal EDS scores (CpG33, P = 0.0003	Results provide further evidence that prenatal adversity can influence imprinted gene methylation, although future studies are needed to unravel the exact mechanism
(Santos et al., 2021)	Human	Descriptive cohort , Locus specific DNA methylation analysis	bisulphite pyrosequencing.	Hypermethylation of *NR3C1* gene resulted in significant associations between IDAS and psychosocial stressors	Several correlations among psychosocial measures,DNA methylation factors and IDAS-II variables were identified. Among the psychosocial measures, everyday discrimination was the most strongly and consistently associated with IDAS-II.
(Wikenius et al., 2019)	Human	Longitudinal epigenome-wide study, DNA methylation	Illumina Infinium HumanMethylation 450 BeadChip.	*CCDC114* (Closest gene), *KLF6* , and *TBR1* .	The analyses revealed no significant genome-wide association between maternal depressive symptoms and infant DNA methylation in the separate analyses and for both timepoints together.
(Perroud et al., 2014)	Human	longitudinal study, Locus DNA methylation analysis	bisulfite conversion, pyrosequencing	*NR3C1* exon *1F* and *NRC32* gene exhibited high methylation in exposed mothers/child in contrast to non exposed groups	This higher methylation status of the *NR3C1* exon 1 F promoter region was associated with reduced GR levels in the blood.
(Montoya-Williams et al., 2017)	Human	Descriptive cohort, locus specific DNA methylation	Illumina HumanMethylation 450 Bead Chip	*IGF2* methylation in maternal blood and birth weight were associated	A strong association was found between newborn birth weight and *IGF2 PC2* methylation in mother's blood (P = 0.0027). For instance, war and rape stress both associated with *IGF1* methylation in cord blood and placenta, whereas war and rape stress associated with *IGF2* methylation in maternal blood

(Cattaneo et al., 2020)	Rats and Human study	Comparative study , miRNA-30a	Methylated DNA immunoprecipitation (MeDIP) analysis and Gene and miRNA expression analyses by Real Time PCR	miR-30a and Neurod1 emerged as potential players for the negative outcomes associated with PNS exposure. also found a significant reduction in the expression of a panel of genes (CAMK2A, c-JUN, LIMK1, MAP2K1, MAP2K2, PIK3CA and PLCG1) that belong to these two biological pathways and are also validated targets of miR-30a, pointing to a down-regulation of these pathways as a consequence of PNS exposure.	found that the methylation status of the miR-30a gene is significantly reduced in the HIP and PFC of adult male and female rats whose mothers had been exposed to stress during the last week of gestation, as compared to controls and also found that miR-30a-5p levels were significantly elevated in the blood of depressed patients who experienced traumatic events early in life, as compared to controls
(Monk et al., 2016)	Human	Prospective study, DNA methylation locus specific	Placental CpG methylation in the three genes was analyzed using 450K Beadchips and bisulfite sequencing; c	increased DNA methylation of *HSD11B2* and *FKBP5* was associated with reductions in a key fetal outcome (coupling) predictive ofinfant neurobehavioral development	Perceived stress (Perceived Stress Scale), but not cortisol, was associated with altered CpG methylation in placentas. In the highest tertile of the Perceived Stress Scale, the Beadchip data revealed modestly elevated methylation of HSD11B2, associated with lower fetal coupling (β=-0.51), and modestly elevated methylation of FKBP5, also with lower fetal coupling (β=-0.47).
(Hompes et al., 2013)	Human	Association study, locus specific DNA methylation	DNA methylation at different loci of the NR3C1 gene, including exon 1B, 1D and 1F, was analyzed in genomic DNA . Bisulfite conversion and Sequenom Epityper MassARRAY	CpG9 was significantly associated with maternal emotional wellbeing. In a multivariable model the proportion of variance in methylation state of F9 explained (PVE) by pregnancy related anxiety was 7.8% (p = 0.023) during T1.	Univariable analyses indicated pregnancy related anxiety to be the strongest psychological parameter throughout pregnancy

(Mansell et al., 2016)	Human	cohort study, DNA methylation	Sequenom MassArray Platform	insulin-like growth factor 2 *(IGF2)* and *H19* , are involved in fetal growth and each is regulated by DNA methylation .	There is strong and consistent evidence of an association between maternal anxiety and decreased IGF2/H19 ICR methylation at the major CpG sites across the region investigated and these associations persisted when accounting for a range of covariates.
(Vidal et al., 2014)	Human	Genome wide association study, DNA methylation	DNA methylation at differentially methylated regions (DMRs) associated with H19, IGF2, MEG3, MEST, SGCE/PEG10, PEG3, NNAT, and PLAGL1 was measured using bisulfite pyrosequencing	*MEST* DMR (2.8% difference, P < 0.01) MEST is a maternally methylated imprinted gene on chromosome 7 that has been implicated in Silver–Russell syndrome (SRS), a syndrome of growth retardation. *Mest* deficiency in mice is associated with abnormal maternal behavior, including deficiencies in rearing of pups as well as growth retardation	elevated stress was associated with higher infant DNA methylation at the MEST DMR (2.8% difference, P < 0.01) after adjusting for PTB.
(Oh et al., 2013)	Human	Descriptive study, locus specific DNA methylation	Sequenom EpiTYPER system	Methylation status in the *NR3C1 1-F* promoter	*NR3C1 1-F* promoter methylation was stronger in infants who had been exposed to low levels of maternal depression in utero.
(Ostlund et al., 2016)	Human	Comparative study, Locus specific DNA methylation	Bisulfite Pyrosequencing DNA Methylation Analysis	*NR3C1* , Exploratory sex-specific analysis revealed a trend-level association between prenatal stress and increased methylation of *NR3C1* exon 1F for female, but not male, infants	. Exploratory sex-specific analysis revealed a trend-level association between prenatal stress and increased methylation of NR3C1 exon 1F for female, but not male, infants
(Kallak et al., 2021)	Human	DNA methylation , Associlation study	Bisulfte conversion of DNA	Several genes, for instance *CRBN* and *MDFIC* , previously shown to be associated with brain development and function where identifed in those who were exposed to SSRIs.	no DM CpG site was identifed in relation to PND alone, diferences in DNA methylation were observed in the cord blood of children whose mothers had PND together with anxiety and also those who were treated with SSRIs.

(Solomonova et al., 2019)	Human	Longitudinal study, DNA mwethylation	vasopressin methylation analysis	AVP methylation	Psychosocial stress was found to moderate the relationship between AVP methylation and sleep disordersduring pregnancy and at postpartum, suggesting that AVP may be in-directly associated with sleep regulation, depending on the underlyinglevels of exposure to psychosocial stress.
(Trump et al., 2016)	Human	Clinical Trial & DNA methylation	Whole genome bisulfite sequencing (WGBS), Validation analysis: MassARRAY and qPCR	*NMUR1/2* , coupled to G alpha q/11 (*GNA11*), *(CACNB4)* , *PPP3R1* , and its downstream target *NFATC3* were methylated.	High maternal stress was associated with an increased risk for persistent wheezing in the child until the age of 5. Deregulated neuroendocrine and neurotransmitter receptor interactions were observed in stressed mothers and their children
(Vangeel et al., 2017)	Human	Cohort, DNA methylation	HumanMethylatio n450 BeadChip and EpiTYPER	DMR in the GABA-B receptor subunit 1 gene *(GABBR1)* revealing the association with pregnancy anxiety particularly in male newborns (most significant CpG P9	Cord blood *GABBR1* methylation was associated with infant cortisol levels in response to a routine vaccination at 4 months old.
(Grasso et al., 2020)	Human	Association study, DNA methylaiton	Bisulfite pyrosequencing and DNA methylation analysis	*FKBP5* rs1360780 genotype and intron 7 methylation *FKBP5* encodes the glucocorticoid receptor (GR) co-chaperone protein (*FKBP51*) and undergoes rapid induction when cortisol activates GRs	Allele-specific associations of methylation with maternal ACEs and prenatal trauma-related symptoms were evident; however, relations differed between mothers and newborns. In mothers carrying the stress sensitive T-allele (CT and TT genotypes), maternal FKBP5 methylation negatively correlated with threat-based ACEs and maternal PTSD symptoms during pregnancy, but not deprivation-based ACEs. In infants homozygous for the C allele (CC genotype), infant FKBP5 methylation positively correlated with